CZECH ACADEMY OF AGRICULTURAL SCIENCES

Czech Journal of ANIMAL SCIENCE

ŽIVOČIŠNÁ VÝROBA

INSTITUTE OF AGRICULTURAL AND FOOD INFORMATION

1

VOLUME 46
PRAGUE 2001
ISSN 1212-1819

CZECH JOURNAL OF ANIMAL SCIENCE

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

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

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

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

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

Acceptance of manuscripts: Two copies of manuscript should be addressed to: Ing. Zdeňka Radošová, Institute of Agricultural and Food Information, Slezská 7, 120 56 Praha 2, Czech Republic, tel.: + 420 2 24 25 34 89, fax: + 420 2 22 51 40 03, e-mail: edit@uzpi.cz.

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

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

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

Přijímání rukopisů: Rukopisy ve dvou kopiích je třeba zaslat na adresu redakce: Ing. Zdeňka Radošová, Ústav zemědělských a potravinářských informací, Slezská 7, 120 56 Praha 2, Česká republika, tel.: + 420 2 24 25 34 89, fax: + 420 2 22 51 40 03, e-mail: edit@uzpi.cz.

Informace o předplatném: Objednávky na předplatné jsou přijímány pouze na celý rok (leden–prosinec) a měly by být zaslány na adresu: Ústav zemědělských a potravinářských informací, vydavatelské oddělení, Slezská 7, 120 56 Praha 2. Cena předplatného pro rok 2001 je 816 Kč.

F 4886

Effects of rapeseed meal and nitrates on thyroid functions in sheep

Vliv extrahovaného řepkového šrotu a dusičnanů na funkci štítné žlázy u ovcí

J. TRÁVNÍČEK[1], V. KROUPOVÁ[1], J. KURSA[1], J. ILLEK[2], R. THÉR[1]

[1]*University of South Bohemia, Faculty of Agriculture, České Budějovice, Czech Republic*

[2]*Ruminant Disease Clinic, Faculty of Veterinary Medicine, Veterinary and Pharmaceutical University, Brno, Czech Republic*

ABSTRACT: Antigoitrogenic effects of increased intake of iodine (250 μg per animal/day) and selenium (150 μg per animal/day) were monitored for 350 days in 24 sheep of the Šumavská breed receiving 4.2 mmol of glucosinolates (GLS) and 4 g of nitrates per animal/day (Tab. 1). The sheep receiving GLS and nitrates without iodine and selenium supplementation developed enlargement of the thyroid gland, low iodine concentration in blood plasma (39.1 μg/l), milk (27 μg/l) and urine (45.1 μg/l), low T_4 level (60.7 nmol/l), high T_3 level (Tab. 3) and a narrow T_3 : T_4 ratio. Neonatal goitre was diagnosed in lambs born to such ewes. Critical periods for the development of hypothyroidism were the breeding period, pregnancy, and lactation. Iodine supplementation had favourable effects (higher blood plasma concentrations of iodine and T_4, normal size of the thyroid gland) in sheep challenged with GLS and nitrates. Selenium concentrations in blood plasma of ewes unsupplemented and supplemented with this mineral reached 23 to 72 μg/l and 200 μg/l, respectively (Tab. 3, Fig. 2). A decrease in the blood plasma concentration of selenium after discontinuing selenium supplementation (264th day of experiment) in the sheep challenged with GLS and nitrates resulted in a transient increase of T_4 concentration in blood plasma. Hypoproteinaemia and hypercholesterolaemia were observed in the last seven weeks of pregnancy and during lactation in ewes receiving no iodine and selenium supplements (Tabs. 6 and 10). No effects of the goitrogens on other haematological indices (urea in blood plasma, haemoglobin, leukocyte count) were observed (Tabs. 7, 8 and 9).

Keywords: sheep; rapeseed meal; nitrates; thyroid gland; iodine; selenium; blood parameters; urine; milk

ABSTRAKT: U čtyř šestičlenných skupin bahnic plemene šumavská ovce (tří pokusných a jedné kontrolní) dlouhodobě zátížených glukosinoláty a dusičnany byl v průběhu 350 dnů sledován antistrumigenní účinek zvýšených dávek jodu a selenu. Bahnice pokusných skupin GIS, GI a G přijímaly denně v krmné směsi 4,2 mmol glukosinolátů (GLS), které byly součástí řepkového extrahovaného šrotu, a 4 g dusičnanů v podobě $NaNO_3$. Bahnicím skupiny GIS byl v týdenních intervalech *s.c.* aplikováván jod (KI) a selen (Na_2SeO_3) v množství, které odpovídá dennímu příjmu 250 μg I a 150 μg Se na bahnici, bahnicím skupiny GI pouze jod v dávce odpovídající 250 μg I na kus. Bahnice kontrolní skupiny IS (n = 6) nebyly zatíženy strumigeny a jejich denní dotace jodu a selenu odpovídala 250 μg I a 150 μg Se na kus (schéma pokusu tab. 1). Jod v moči, mléku a v krevní plazmě byl stanoven po alkalické digesci spektrofotometricky (Bednář *et al.*, 1964), T_3 a T_4 radioimunologicky, selen atomovou absorpcí. Bahnice zatížené glukosinoláty a dusičnany bez doplňku jodu (G) vykazovaly za celou dobu experimentu nejnižší průměrný obsah jodu v krevní plazmě (tab. 3): skupina G 39,1 ± 5,5 μg /l, skupiny GIS, GI a IS 46,3 až 52,0 μg/l ($P < 0,01$). Největší pokles byl v období zapouštění, v první polovině gravidity a v počátku laktace (obr. 1). Výraznější rozdíly (tab. 3) byly v obsahu jodu v moči: skupina G 45,1 ± 28,8 μg/l, skupina GIS 88,8 ± 42,8, skupina GI 80,1 ± 27,7, skupina IS 107,4 ± 54,5 μg/l ($P < 0,01$) a v mléku: skupina G 27,0 ± 15, skupiny GIS a GI 70 až 101 μg/l a skupina IS 198,0 ± 8 μg/l ($P < 0,01$). Vyšší koncentrace jodu v plazmě skupin GIS, GI a IS (tab. 3) byla provázena vyšší hladinou T_4 (78,1 až 89,2 nmol/l). Nižší koncentrace T_4 (60,7 ± 14,5 nmol/l) a vyšší koncentrace T_3 (3,3 ± 0,7 nmol/l) u skupiny G v porovnání s ostatními skupinami ($P < 0,01$) jsou projevem hypotyreózy v souvislosti se sekundární jodopenií. Vedle těchto projevů byla u bahnic zjištěna zvětšená štítná žláza a u narozených jehňat

Supported by Grant Agency of the Czech Republic (Grant No. 524/97/0654) and Ministry of Agriculture of the Czech Republic (Grant NAZV No. EP 9269/99) and by Ministry of Education of the Czech Republic (Grant CEZ No. J 06/122200002).

neonatální struma. Dynamika jodu (obr. 1) a hormonů štítné žlázy (tab. 4 a 5) včetně zúženého poměru $T_3 : T_4$, dokumentují zvýšené riziko hypotyreózy v průběhu zapouštění, gravidity, porodu a laktace. Vyšší koncentrace jodu a T_4 u bahnic skupin GIS a GI (tab. 3) dokazují pozitivní uplatnění doplňku jodu při kompenzaci antityreoidálního efektu souběžně působících glukosinolátů a dusičnanů. Koncentrace selenu se v krvi bahnic, které byly bez jeho pravidelné dotace, pohybovala v rozmezí 23 až 72 μg/l, u bahnic s jeho doplňkem překročila 200 μg/l (tab. 3, obr. 2). Ukončení aplikace selenu (264. den pokusu) provázel pokles jeho koncentrace v krvi a u bahnic zatížených glukosinoláty a dusičnany (skupina GIS) přechodné zvýšení T_4 v plazmě. V poslední třetině gravidity a v průběhu laktace byl u bahnic zatížených strumigeny bez suplementace I a Se (skupina G) zaznamenán pokles proteinemie a vzestup cholesterolemie (tab. 6 a 10). Ostatní krevní parametry (močovina v plazmě, hemoglobin, počty leukocytů) nebyly zvolenou formou strumigenní zátěže jednoznačně ovlivněny (tab. 7, 8 a 9).

Klíčová slova: ovce; řepkový extrahovaný šrot; dusičnany; štítná žláza; jod; selen; krevní parametry; moč; mléko

INTRODUCTION

The current intensification of agricultural production increases the significance of goitrogenic activity of glucosinolates and nitrates. Glucosinolates (GLS) are present above all in plants of the mustard family *(Brassicaceae)*, in particular in their seeds (Maas, 1987). Cabbage and rape are topical sources of GLS for farm animals. So far 120 GLS have been identified in rape. Enzymatic hydrolysis or chemical degradation converts them into goitrogenic isothiocyanates (Herzig *et al.*, 1999a). Thus, the enzyme myrosinase converts progoitrine present in rape into the strongly goitrogenic goitrine which inhibits the transport of iodine in the thyroid gland and thereby the synthesis of thyroxine (Velíšek and Hrnčiřík, 1996; Schöne, 1993). The content of GLS in seeds of the most widely cultured double zero ("00") varieties of rape ranges from 8 to 32 mmol/g (Pajtáš, 1999) and can reach up to 100 mmol/g in single zero ("0") varieties (Herzig *et al.*, 1999a).

Rapeseed meal (RM) is recommended as a protein-rich component of concentrates for dairy cows. Its proportion can amount to 25% (Pajtáš, 1999). Comparative trials with rapeseed cake or RM and soybean meal included at 25% into diets for lactating cows did not demonstrate any depressing effects on their performance. The authors who claim the performance enhancing effects of rape products recommend to complete such diets with adequate amounts of macroelements, above all calcium and magnesium, and trace elements, such as copper, manganese, zinc, selenium, molybdenum and above all iodine.

Although ruminants are believed to be more tolerant to the toxic effects of GLS due to their ruminal degradation, several authors pointed out the risk of feeding rapeseed meal to pregnant females (Rudert and Oliver, 1976) or calves and lambs (Herzig *et al.*, 1999a), in which the ruminal detoxification of isothiocyanates is less effective and the requirements for thyroid hormones are higher. Besides isothiocyanates, the cumulation of iodine in the thyroid gland by inhibition of the active transport of iodine through membranes of follicular cells is reduced also by nitrates and other univalent anions (Pandav and Rao, 1997; Písaříková *et al.*, 1996). Factors influencing iodine utilisation include also selenium which, in the form of selenium-containing enzymes, controls the production of thyroid hormones and their final transformation at the tissue level (Köhrle, 1996) and, as a component of peroxidases, participates in the elimination of peroxides thus compensating their destructive effect on thyreocytes (Corvilain *et al.*, 1993). The goitrogenic effects of GLS and nitrates can be compensated by increased intake of iodine (Schöne *et al.*, 1991; Trávníček *et al.*, 1999).

Increased intake of GLS or nitrates is known to result in enlargement of the thyroid gland and changes in its microscopic structure. Information on their effects on thyroid hormones is less frequent and sometimes contradictory (Barry *et al.*, 1983; Schöne, 1993; Rao and Lakshmy, 1995; Herzig *et al.*, 1999a).

In addition to their direct effect on the thyroid gland and its activity, GLS and nitrates can influence directly or via changes in thyroid hormone levels also other functions, such as methaemoglobinaemia or they affect the detoxifying activity of hepatocytes (Duncan and Milne, 1993). The multiple effects of GLS and nitrates can be assessed from parameters of proteosynthesis (plasmatic total protein and urea, haemoglobin), lipid metabolism (cholesterol) and immunocompetence (leukocytes).

The objective of this study was to assess goitrogenic effects of RM and nitrates including the compensatory role of iodine and selenium supplementation, and to investigate the effects of the above factors on thyroid functions, indirect indicators of proteosynthesis, lipid metabolism, and leukocyte count.

MATERIAL AND METHODS

Twenty-four ewes of the Šumavská breed were divided into four groups of six (GIS, GI, G, IS). The groups are designated according to the intake of goitrogen and supplementation with iodine or selenium. The design of the experiment, diet formulation, and data on iodine and

selenium supplementation are given in Tab. 1. Composition of concentrates is given in Tab. 2.

The sheep were housed in groups in a deep-litter barn, fed twice a day, and had free access to crockery troughs with potable water supplied from a public source.

mined by gas chromatography after derivatisation to silyl compounds (Zukalová and Vašák, 1978). The group IS received the concentrate A in which RM was replaced by oats meal and urea. The experiment was started by a 7-day adaptation period when the animals received one

Table 1. Goitrogenic effect of rapeseed meal and nitrates in ewes and their progeny – experimental design

Group	Initial average weight (kg)	Feed ration (animal/day)	Goitrogen	Supplementation
GIS $n = 6$	41.0	hay 1.4 kg concentrate B 287 g lick	4.2 mmol GLS (RM) 4 g $NaNO_3$	iodine 250 μg as a KI solution once per week *s.c.* selenium 150 μg as Selevit once per week *s.c.*
GI $n = 6$	43.8	hay 1.4 kg concentrate B 287 g lick	4.2 mmol GLS (RM) 4 g $NaNO_3$	iodine 250 μg as a KI solution once per week *s.c.*
G $n = 6$	44.5	hay 1.4 kg concentrate B 287 g lick	4.2 mmol GLS (RM) 4 g $NaNO_3$	0
IS $n = 6$	43.3	hay 1.4 kg concentrate A 287 g lick	0	iodine 250 μg as a KI solution once per week *s.c.* selenium 150 μg as Selevit once per week *s.c.*

GLS = glucosinolates
RM = rapeseed meal

Table 2. Composition of concentrates

Ingredient	A (kg)	B (kg)
Calcium carbonate ($CaCO_3$)	4.8000	4.8000
Sodium chloride (NaCl)	1.8000	1.8000
Zinc oxide (ZnO)	0.0087	0.0087
Copper sulphate ($CuSO_4 .5 H_2O$)	0.0018	0.0018
Flour	9.0000	9.0000
Sodium nitrate ($NaNO_3$)	0.0000	1.9000
Urea	1.5000	0.0000
Oats meal	82.9000	0.0000
Rapeseed meal	0.0000	82.5000

In all the groups, the major ingredient of the diet was meadow hay harvested in the area of the sheep origin. Hay was completed with the concentrates A and B prepared in an experimental feed mill of the Central Institute for Supervising and Testing in Agriculture, Lysá nad Labem. Vitamin additives Combinal A forte, Combinal D forte, and Combinal E forte were administered in drinking water. Moreover, feeding carrot was offered and iodine-free lick salt was available.

The sheep of the groups GIS, GI, and G received daily the concentrate B containing rapeseed meal (RM – 82.5%) and sodium nitrate (NO_3 – 1.9%). The dose was 287 g per animal/day and corresponded to a daily intake of 4 g of sodium nitrate and 4.2 mmol of glucosinolates per animal/day. The content of glucosinolates was deter-

half of the concentrates. Additional sources of iodine and selenium for the sheep of the groups GIS, GI, and G were a potassium iodide solution and Selevit inj. ad us. vet. containing anhydrous sodium selenite as the active substance. The supplements were administered subcutaneously at weekly intervals in doses corresponding to 250 μg of iodine and 150 μg of selenium per animal. The supplementation vith selenium was interrupted in regard to its increased concentration in blood plasma (264th day of the experiment).

The ewes were bred naturally from day 81 to day 187 using two breeder rams tested for fertility. The number and percentage of pregnant ewes: group GIS (6/100), group GI (6/100), group G (6/100) and group IS (5/83.3). This experiment and observation were conducted for 350 days.

Blood samples were collected from *v. jugularis* into test tubes containing heparin as the anticoagulant. Urine samples were obtained by catheterization.

Blood plasma, urinary, and milk iodine was determined after alkaline digestion by the spectrophotometric method of Sandell-Kolthoff as modified by Bednář *et al.* (1964). Conventional methods were used for determination of the remaining blood components: haemoglobin as haemoglobin cyanide, leukocyte count haemocytometrically, blood plasma cholesterol by reaction with acetic anhydride, triiodothyronine (T_3) and thyroxine (T_4) radioimmunologically using commercial kits (Immunotech, Prague), urea colorimetrically using the Biola-test (Lachema, Brno), and blood selenium by atomic absorption after

mineralisation by the hydride technique (Unicam). Significance of differences in mean values was assessed using the *t*-test and dependence between hormones (T_4, T_3) and iodine concentrations in blood plasma was tested by correlation analysis in both cases using the software STAT-plus (Matoušková *et al.*, 1992).

RESULTS

No clinical abnormalities were detected by adspection and palpation of the thyroid glands except for three ewes of the group G. The latter three ewes developed a slight enlargement making the thyroid gland palpable on experimental Day 56. Typical goitre developed in the lambs. One female lamb of the group G, born with a low viability and live weight, was lethargic and developed thyroid enlargement palpable as two distinct painless, oval rigid, and elastic structures with sizes of 3–4 x 2 cm.

Favourable effects of regular weekly supplementation of ewes fed a diet containing nitrates and glucosinolates and supplemented with iodine and selenium are apparent from both dynamics (Fig. 1) and mean iodine concentrations in blood plasma (Tab. 3). Ewes of the control group G showed repeatedly the lowest iodine concentrations in blood plasma for almost the whole experimental period. Compared with the groups GIS, GI, and IS, maximum differences were observed during the breeding and the first half of the gestation periods and in lactating ewes. Mean iodine concentration in blood plasma for the whole experimental period (Tab. 3) in the group G was significantly lower than in any of the other groups ($P < 0.01$).

Although the urinary iodine output is highly density-dependent, its concentration in the group G was lower by 35–62.3 μg I/l in comparison with the other groups (Tab. 3). The iodine concentration in milk, which is a significant indicator of iodine intake, dropped in the group G during the experimental period down to 27 ± 15 μg/l. Compared with the other groups, in which the concentrations ranged between 70 and 198 μg/l, the difference was significant ($P < 0.01$).

The higher iodine concentrations in blood plasma in the groups GIS, GI, IS were accompanied by higher levels of plasma T_4 ($P < 0.01$, Tab. 3). The correlation between iodine and T_4 concentrations was confirmed by the coefficient $r = 0.50$ ($P < 0.01$).

On the other hand, the group G showed higher concentrations of triiodothyronine (T_3), particularly in pregnant and lactating ewes (Tab. 4). Compared with the remaining groups, the difference in mean concentrations of T_3 for the whole experimental period was significant ($P < 0.05$; 0.01; Tab. 3). The correlation between T_3 and iodine concentrations in blood plasma was insignificant ($r = 0.13$).

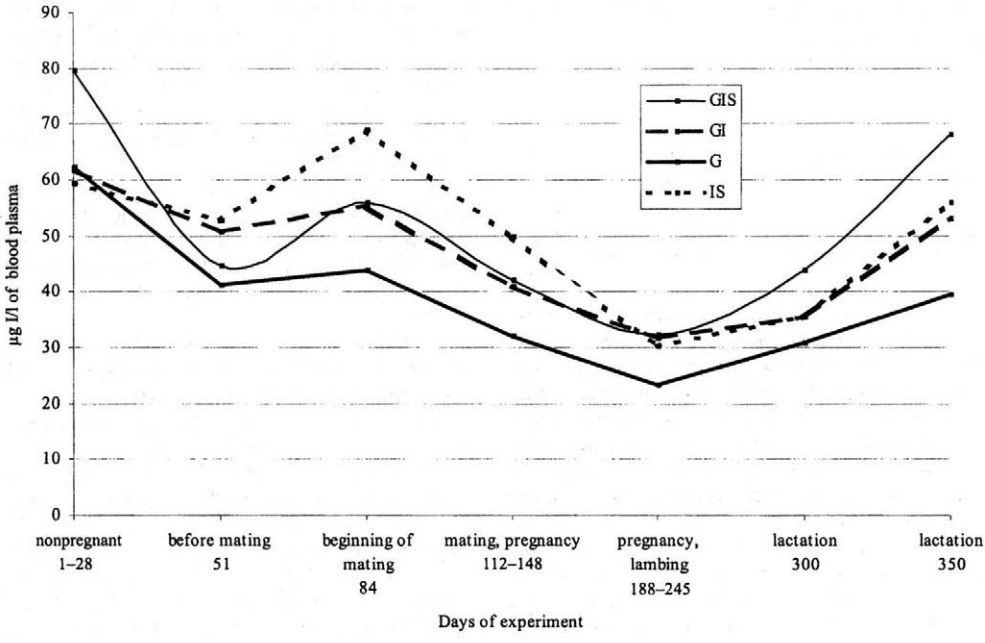

Figure 1. Concentration of iodine in blood plasma (μg/l) in the experiment

Table 3. The mean content of iodine in plasma, urine, and milk; of thyroxine and triiodothyronine in plasma; of selenium in blood

Concentration		Number of examinations	Group GIS		Group GI		Group G		Group IS		*T*-test
			$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	
Plasma iodine	µg/l	60	52.0[1]	7.9	46.3[2]	12.5	39.1[1, 2, 3]	5.5	49.2[3]	10.7	[1, 2, 3]($P < 0.01$)
Urine iodine	µg/l	60	88.8[1, 4]	42.8	80.1[2, 5]	27.7	45.1[1, 2, 3]	28.8	107.4[3, 4, 5]	54.5	[1, 2, 3]($P < 0.01$) [4,5]($P < 0.05$)
Milk iodine	µg/l	12	70.0[1, 4]	16.0	101.0[2, 5]	41.0	27.0[1, 2, 3]	15.0	198.0[3, 4, 5]	8.0	[1, 2, 3, 4, 5]($P < 0.01$)
Plasma T_4	nmol/l	60	82.6[1]	23.1	78.1[2]	30.4	60.7[1, 2, 3]	14.5	89.2[3]	35.1	[1, 2, 3]($P < 0.01$)
Plasma T_3	nmol/l	60	3.1[2]	0.6	3.0	0.8	3.3[1]	0.7	2.8[1, 2]	0.7	[1]($P < 0.01$) [2]($P < 0.05$)
Blood selenium	µg/l	60	202.3[1, 2]	111.9	45.2[2, 3, 5]	13.1	53.9[1, 3, 4]	16.9	186.1[4, 5]	84.6	[1, 2, 3, 4, 5]($P < 0.01$)

Table 4. Concentration of T_3 in blood plasma (nmol/l) in the experiment

Days of experiment	Stage of reproductive cycle	Number of examinations	Group GIS		Group GI		Group G		Group IS		*T*-test
			$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	
1–28	nonpregnant	12	3.2[2]	0.5	3.5	1.0	3.9[1, 2]	0.5	2.8[1]	0.4	[1, 2]($P < 0.01$)
51	before mating	6	2.2	0.4	2.4	0.6	2.6	0.4	2.7	0.5	
84	beginning of mating	6	4.7	1.5	4.5	1.4	4.2	1.2	4.1	0.8	
112–148	mating, pregnancy	12	3.5	0.7	3.3	1.1	3.6	0.7	3.6	1.0	
188–245	pregnancy, lambing	12	2.7[1]	0.5	2.6[2]	0.5	3.3[1, 2, 3]	0.4	2.3[3]	0.6	[1, 2, 3]($P < 0.01$)
300	lactation	6	3.1	0.7	2.9	0.9	3.3	1.4	2.3	0.7	
350	lactation	6	2.0	0.2	2.1	0.4	2.4	0.3	1.9	0.6	

The $T_3 : T_4$ ratio changed from 84th to 148th day of the experiment (breeding period, pregnancy) from 1 : 16–41 to 1 : 15–23. In the group G, the closer ratio persisted up to the end of the experiment (1 : 16–20). In the groups GIS, GI and IS the $T_3 : T_4$ ratio returned to the initial value (1 : 25–40) in the second half of the experimental period.

Blood selenium concentrations in the unsupplemented groups G and GI did not exceed 70 µg/l. On the other hand, the concentrations increased to more than 200 µg/l after Day 60 in the groups GIS, IS (Fig. 2). A marked decrease was observed as soon as the supplementation was discontinued on day 264. A difference in blood selenium concentrations between the supplemented groups GSI and IS was insignificant and the same applied to the difference between the unsupplemented groups GI and G (Tab. 3). Worth mentioning is a transient increase in T_4 concentration in the group GSI after the supplementation was discontinued (Tab. 5).

Table 5. Concentration of T_4 in blood plasma (nmol/l) in the experiment

Days of experiment	Stage of reproductive cycle	Number of examinations	Group GIS		Group GI		Group G		Group IS		*T*-test
			$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	
1–28	nonpregnant	12	88.2[1]	2.2	99.3[2]	17.7	62.0[1, 2, 3]23.5		105.2[3]	16.0	[1, 2, 3]($P < 0.01$)
51	before mating	6	91.1	15.8	68.9[1]	22.8	78.7[2]	16.5	102.8[1, 2]	8.8	[1]($P < 0.01$) [2]($P < 0.05$)
84	beginning of mating	6	84.0	24.0	81.0	18.6	62.9	12.4	81.0	30.5	
112–148	mating, pregnancy	12	68.5	0.6	76.5	14.1	64.5	17.6	68.5	15.1	
188–245	pregnancy, lambing	12	67.8[2]	15.6	72.7	28.9	53.0[1, 2]	15.4	76.8[1]	16.1	[1]($P < 0.01$) [2]($P < 0.05$)
300	lactation	6	121.8[1]	17.9	64.8	28.3	57.0[1]	16.6	69.6	27.2	[1]($P < 0.01$)
350	lactation	6	80.8[1]	17.7	69.0	24.0	47.7[1]	16.5	74.7	25.8	[1]($P < 0.01$)

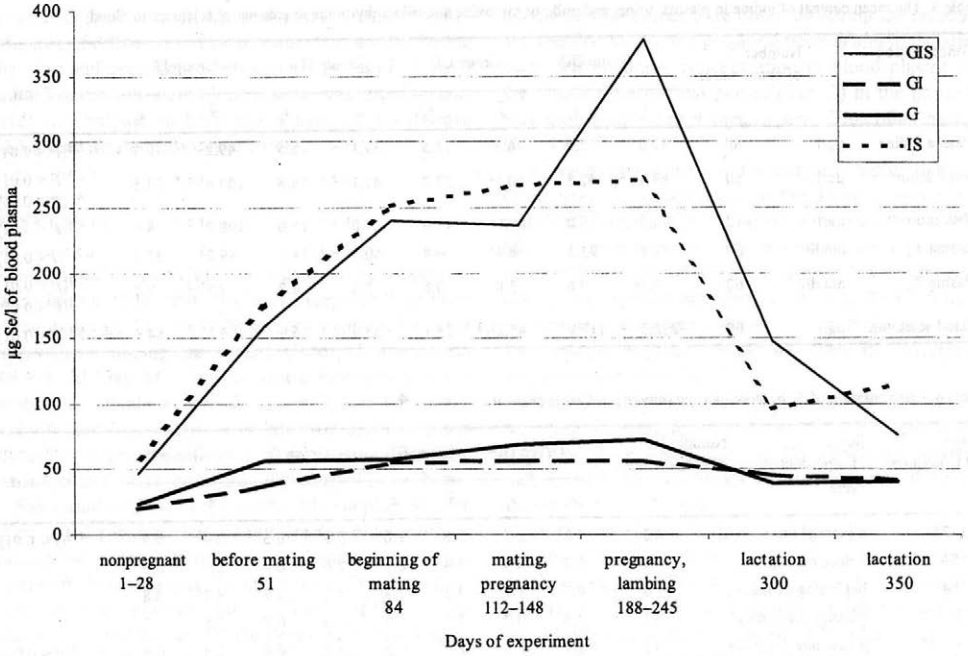

Figure 2. Concentration of selenium in blood plasma (µg/l)

Individual and partial mean total protein concentrations varied considerably during the continuing growth and development of the experimental animals (Tab. 6). A decrease to 60 g/l was observed during the last seven weeks of pregnancy and lactation. This decrease was most marked in pregnant ewes of the group G (49.7 ± 8.2 g/l; $P < 0.01$).

Urea concentration in blood plasma varied within a range of 5.2 to 10.0 mmol/l during the experimental period (Tab. 7). Higher concentrations were observed in the groups GIS, GI, and G.

In all the groups, the concentration of haemoglobin was higher than 100 g/l. Lower values were found in all the four groups during the first 7 weeks of the experiment and in lactating ewes in the last phase of the experiment (Tab. 8). Mean haemoglobin concentrations of the groups GIS, GI, and G were alike. A higher concentration was observed in the group IS from the beginning of the experiment.

Leukocyte counts (Tab. 9) differed considerably both between the groups and within the groups. No significant differences were found in mean values, however.

The blood plasma cholesterol concentration (Tab. 10) decreased during pregnancy and lactation in the group GIS, GI and IS to 0.9–1.5 mmol/l. In the group G the concentration remained at 1.5 to 1.8 mmol/l also in these phases of the experiment ($P < 0.01$; 0.05).

Table 6. Concentration of total proteins in blood plasma (g/l) in the experiment

Days of experiment	Stage of reproductive cycle	Number of examinations	Group GIS		Group GI		Group G		Group IS		T-test
			$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	
1–28	nonpregnant	12	68.3	10.2	62.4	7.7	64.4	11.5	67.0	7.8	
51	before mating	6	66.8	7.8	77.4	8.9	67.7	5.4	70.9	8.9	
84	beginning of mating	6	66.7	14.6	73.4	20.3	67.4	19.7	71.7	21.8	
112–148	mating, pregnancy	12	65.5	6.5	69.6^{1}	5.3	$61.2^{1,2}$	6.8	70.1^{2}	8.4	^{1,2} ($P < 0.01$)
188–245	pregnancy, lambing	12	60.1^{1}	2.5	60.4^{3}	9.3	$49.7^{1,2,3}$	8.2	61.0^{2}	8.2	^{1,2,3} ($P < 0.01$)
300	lactation	6	65.0	8.6	62.3	3.2	58.3	2.1	61.1	3.9	
350	lactation	6	62.3	4.3	61.9	6.6	59.5	2.7	60.6	3.1	

Table 7. Concentration of urea in blood plasma (mmol/l) in the experiment

Days of experiment	Stage of reproductive cycle	Number of examinations	Group GIS		Group GI		Group G		Group IS		T-test
			$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	
1–28	nonpregnant	12	6.6 [1]	1.1	6.4 [2]	0.6	6.2 [3]	0.4	5.3 [1,2,3]	0.9	[1,2,3] ($P < 0.01$)
51	before mating	6	7.1 [3]	1.0	7.9 [1]	0.9	8.4 [2,3]	0.6	6.4 [1,2]	0.5	[1,2] ($P < 0.01$) [3] ($P < 0.05$)
84	beginning of mating	6	8.0	1.3	9.8 [1]	0.8	9.0 [2]	0.7	7.8 [1,2]	1.1	[1] ($P < 0.01$) [2] ($P < 0.05$)
112–148	mating, pregnancy	12	8.2 [1]	0.8	8.8 [2]	1.0	8.8 [3]	0.8	7.0 [1,2,3]	0.7	[1,2,3] ($P < 0.01$)
188–245	pregnancy, lambing	12	6.1 [3]	1.2	7.0 [1]	1.3	6.8 [2]	1.2	5.2 [1,2,3]	0.9	[1,2] ($P < 0.01$) [3] ($P < 0.05$)
300	lactation	6	7.2	1.3	8.5 [1]	1.3	7.4	0.8	6.4 [1]	0.9	[1] ($P < 0.01$)
350	lactation	6	8.4 [1]	1.1	10.0 [2]	1.3	9.1 [3]	1.5	5.7 [1,2,3]	1.1	[1,2,3] ($P < 0.01$)

Table 8. Concentration of haemoglobin in blood (g/l) in the experiment

Days of experiment	Stage of reproductive cycle	Number of examinations	Group GIS		Group GI		Group G		Group IS		T-test
			$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	
1–28	nonpregnant	12	106.3	23.4	109.6	10.3	108.7	7.5	113.1	7.9	
51	before mating	6	109.8	3.7	110.0	5.8	108.4	6.8	114.3	1.9	
84	beginning of mating	6	115.2[1]	3.7	115.4	10.9	112.6[2]	5.8	125.1[1,2]	6.3	[1,2] ($P < 0.01$)
112–148	mating, pregnancy	12	117.3	7.7	113.4	9.4	113.2	6.5	115.3	7.9	
188–245	pregnancy, lambing	12	118.1	6.7	119.0	4.9	122.0	5.8	119.0	7.0	
300	lactation	6	118.7	9.0	112.3	8.7	117.3	8.4	121.6	11.7	
350	lactation	6	111.2	10.2	100.4[1]	9.6	110.4	9.6	114.8[1]	11.7	[1] ($P < 0.05$)

Table 9. Count of leukocytes in blood (G/l) in the experiment

Days of experiment	Stage of reproductive cycle	Number of examinations	Group GIS		Group GI		Group G		Group IS		T-test
			$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	
1–28	nonpregnant	12	7.1	1.6	7.1	1.5	7.3	1.1	6.9	1.8	
51	before mating	6	6.4[1,3]	1.3	8.9[1,2]	1.3	8.2[3,4]	1.2	6.8[2,4]	0.5	[1,2] ($P < 0.01$) [3,4] ($P < 0.05$)
84	beginning of mating	6	5.8	0.8	7.6	2.2	6.7	2.1	5.8	1.4	
112–148	mating, pregnancy	12	6.6	1.2	7.6	1.4	5.8	1.8	6.9	1.8	
188–245	pregnancy, lambing	12	6.2	1.3	7.0	1.5	6.2	1.3	6.4	1.5	
300	lactation	6	7.0	2.0	6.7	1.8	6.6	1.3	5.9	1.3	
350	lactation	6	7.2	1.9	5.5	2.6	6.4	0.8	5.8	1.0	

Table 10. Concentration of cholesterol in blood plasma (mmol/l) in the experiment

Days of experiment	Stage of reproductive cycle	Number of examinations	Group GIS		Group GI		Group G		Group IS		T-test
			$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	
1–28	nonpregnant	12	1.6[1,2]	0.3	1.3[1,3]	0.2	1.7[3,4]	0.4	1.3[2,4]	0.2	[1,2] ($P < 0.01$) [3,4] $P < 0.05$)
51	before mating	6	1.6	0.3	1.8	0.3	1.9	0.4	1.9	0.2	
84	beginning of mating	6	2.2	0.4	1.8	0.5	1.8	0.3	2.0	0.3	
112–148	mating, pregnancy	12	1.7[3]	0.4	1.7[2]	0.3	2.0[1,2,3]	0.3	1.5[1]	0.2	[1] ($P < 0.01$) [2,3] ($P < 0.05$)
188–245	pregnancy, lambing	12	1.3[1]	0.3	1.4[2]	0.2	1.8[1,2,3]	0.3	1.3[3]	0.3	[1,2,3] ($P < 0.01$)
300	lactation	6	1.5	0.3	1.3[1]	0.1	1.6[1]	0.2	1.5	0.2	
350	lactation	6	1.0[2]	0.4	1.1[3]	0.3	1.5[1,2,3]	0.3	0.9[1]	0.2	[1] ($P < 0.01$) [2,3] ($P < 0.05$)

DISCUSSION

The significantly higher concentrations of blood plasma iodine in the groups GIS, GI, IS (Tab. 3) were apparently due to its increased intake in the form of regular parenteral administration. Compared with the results published by Rogers (1992) and McCoy *et al.* (1997), the blood plasma concentrations of iodine found in the group G must be regarded as low and indicative of insufficient content in the basic ration. Similar values not exceeding 20 μg/l were reported by Groppel *et al.* (1987) for sheep fed a diet containing only 0.05 to 0.13 mg iodine per 1 kg dry matter for approximately the same period. An increase in the concentration of dietary iodine to 0.4 mg per 1 kg dry matter, corresponding to the recommended range of 0.2 to 0.5 mg per 1 kg dry matter (Jenkins and Hidiroglou, 1990), resulted in an increase of blood plasma iodine concentration to 67 μg/l (Groppel *et al.*, 1987).

Even larger between-the-groups differences were found in the concentrations of urinary and milk iodine (Tab. 3). The mean urinary iodine concentration of 45.1 μg/l in the group G corresponded to values reported by Herzig *et al.* (1999b) for cows fed an iodine-deficient diet consisting of meadow hay, dried alfalfa and grain meal. Considering the naturally higher milk iodine concentration in sheep, the mean value found in the group G (27 μg/l) can be interpreted as a sign of marked iodopoenia. Groppel (1991) classified as iodine-deficient animals those with milk iodine concentration as high as 62 μg/l.

A discrepancy was found between approximately the same concentration of blood plasma iodine and different urinary and milk iodine concentrations in ewes of the groups GIS, GI, IS ($P < 0.01$, Tab. 3, Fig. 1). The lower concentration of milk iodine in the groups GIS and GI can be explained as a result of inhibition of active iodine transport into the mammary gland due to the competitive action of nitrates and GLS, as described for the thyroid gland by Písaříková *et al.* (1996). A similar decrease in milk iodine concentration in dairy cows fed GLS-containing rapeseed cake was reported by Suchý *et al.* (1998). The dysbalance between iodine intake and excretion in animals in which iodine utilisation by the thyroid gland is limited due to the goitrogenic effect of GLS and nitrates allows speculations on iodine "retention" in other tissues or alternative ways of its excretion.

The significant decrease in blood plasma T_4 concentration in the group G (Tabs. 3, 5) reflects the goitrogenic effect of GLS and nitrates. The higher concentrations of T_4 in blood plasma of ewes of the groups GIS and GI demonstrate the compensatory antigoitrogenic effect of iodine supplementation. Similar results were also published by Ehlers *et al.* (1994) and Kvíčala *et al.* (1997).

The lower iodine and T_4 and higher T_3 concentrations in the group G (Tabs. 3, 4 and 5, Fig. 1) indicated that iodine supply was inadequate for a normal function of the thyroid gland (Janssen *et al.*, 1994). Under such conditions, the thyroid gland preferably synthesises T_3 in which the iodine content is lower than in T_4. In addition to the above signs of hypothyroidism, a moderate enlargement of the thyroid gland was found in three ewes by palpation already in the second week of the experiment and neonatal goitre developed in their progeny. The dynamics of blood plasma iodine concentration and thyroid hormones (Fig. 1, Tabs. 4 and 5), including the narrow $T_3 : T_4$ ratio, are evidences of an increased risk of thyroid hyperfunction under the influence of steroid hormones during the breeding period, pregnancy, parturition and lactation period (Bekeová *et al.*, 1994). The signs of hypothyroidism in the group G, including a T_4 decrease and low iodine concentration in blood plasma, milk and urine, only point out a danger of contribution of further adverse factors, including glucosinolates and nitrates, to the aetiology of thyreopathies under challenging metabolic situations. A similar marked decrease in T_4 (< 20 nmol/l) and concurrent increase in T_3 (≥ 3 nmol/l) were found by Groppel *et al.* (1987) in pregnant goats suffering from a marked iodine deficiency.

Blood selenium concentrations in sheep of the groups G and GI (Tab. 2, Fig. 2) corresponded to the half-level of the normal values of 120 to 150 μg/l as defined by Stowe and Herd (1992) as the maximum. On the other hand, the dynamics of selenium concentrations in blood plasma of sheep of the groups GIS and IS indicated that the parenteral weekly dose was excessive. Discontinuation of the selenium supplementation was followed by a decrease in blood selenium concentration and, in the group GIS, a transient increase in blood plasma T_4 concentration (Fig. 2). The sudden decrease in blood selenium concentration in the sheep adapted to the previous excessive supply could have resulted in a transient decrease in the activity of 1,5' deiodase and in a conversion of T_4 at both the thyroid gland and the peripheral levels (Kreze *et al.*, 1993).

The effects of goitrogens (GLS + nitrates) and selenium and iodine supplementation on haematological indices are shown in Tabs. 6 to 10. The effects of goitrogens in the group G included a decrease in total protein and an increase in cholesterol concentrations in blood plasma. The subnormal protein concentrations in late pregnancy and lactation (Richter *et al.*, 1983) in this group can be associated with the thyroid hormone deficiency and a decrease in the proteosynthetic activity resulting therefrom (Reeds, 1987). Blood cholesterol concentrations (Tab. 10) were within the normal range (Jagoš and Bouda, 1981) in all the groups. However, differences between the groups appeared in late pregnancy and in the lactation period. The higher cholesterolaemia in the group G was a result of depressed activity of the thyroid gland affecting the metabolism of lipids (Dory and Roheim, 1981; Bíreš *et al.*, 1996). In addition to depressed proteosynthesis an alteration in hepatic cholesterol metabolism can be considered in the sheep receiving goitrogens

without iodine and selenium supplementation. Findings indicative of adverse effects of goitrogens on proteosynthesis and lipid metabolism stress the importance of mineral and trace element, particularly iodine supplementation (Šimek *et al.*, 1999) of goitrogenic diets also for productive efficiency.

Haemoglobin concentrations (Tab. 8) were at the lower limit of the normal range, as given for sheep by Richter *et al.* (1983), in all the groups receiving goitrogens irrespective of iodine and selenium supplementation. The significant decrease in haemoglobin concentration in lactating sheep of the group GI apparently resulted from the fact that natality was higher (150%) than in the other groups (Kursa *et al.*, 2000).

Mean leukocyte counts did not exceed the physiological range as defined by Richter *et al.* (1983). Due to large individual variability, the differences between the groups were not significant. Since the intake of glucosinolates and nitrates had no marked effect on leukocyte count, no impact on the immune system is assumed. The finding of unaltered leukocyte count can also be interpreted as an indirect evidence of normal bone marrow activity.

Although the concentration of urea in blood plasma fluctuated considerably, the limits of the physiological range, as defined by Bock and Polach (1994), were not exceeded. Higher mean values found in the groups GSI, G, and GI can be explained as a consequence of increased intake of rapeseed meal proteins which undergo ruminal degradation.

REFERENCES

Barry T.N., Duncan W.A., Sadler K.R., Tillar A.D. (1983): Iodine metabolism and thyroid hormone relationships in growing sheep fed on kale (*Brassica oleracea*) and ryegrass (*Lolicon perenne*) – clover (*Trifolium repens*) fresh forage diets. Brit. J. Nutr., *49*, 41–253.

Bednář B., Rőhling S., Vohnout S. (1964): Příspěvek ke stanovení proteinového jodu v krevním séru. Českoslov. Farm., *13*, 203–209.

Bíreš J., Bartko P., Weissová T., Matišák T., Michna A., Bírešová M. (1996): Clinical and metabolic response of goats suffering from iodopenia to potassium iodide application. Vet. Med. – Czech, *41*, 177–182.

Bekeová E., Krajničáková M., Hendrichovský V., Maraček I. (1994): The effects of long-acting oxytocin, GnRH and FSH administration on thyroxin, triiodothyronin, oestradiol 17-β and progesterone levels as well as conception rates in postpartum eves. Anim. Reprod. Sci., *37*, 311–323.

Bock U., Polach (1994): Směrné hodnoty důležitých laboratorních vyšetření pro domácí zvířata. 1. vyd. Vetpres – vydavatelství a. s. Biopharm – VÚBVL, Jílové u Prahy. 127 pp.

Corvilain B., Contempré B., Longombré A.O. (1993): Selenium and the thyroid: how the relationship was established. Amer. J. Clin. Nutr., *57*, 244–248.

Dory L., Roheim P.S. (1981): Rat plasma lipoproteins and apolipoproteins in experimental hypothyroidism. J. Lipid Res., *22*, 287–296.

Duncan A.J., Milne J.A.(1993): Effects of oral administration of brassica secondary metabolites, allyl cyanide, allyl isothiocyanate and dimethyl disulphide, on the voluntary food intake and metabolism of sheep. Brit. J. Nutr., *70*, 631–645.

Ehlers K., Wemheuer W., Paufler S. (1994): FT_4- und T_4- Serumspiegel von Milchkühen nach Thiocyanatbelastung mittels 00-Rapsextraktionsschrot bei unterschiedlicher Jodversorgung. In: Anke M. *et al.*: Defizite und Überschüsse an Mengen- und Spurenelementen in der Ernährung. 14. Arbeitstagung Mengen- und Spurenelemente 1994, Friedrich-Schiller-Universität, Jena. 628–636.

Groppel B., Anke M., Jahreis G., Köhler B., Scholtz E. (1987): Jodversorgung und Jodstatus des Wiederkäuers 7. Mitteilung: Einfluus der Jodversorgung auf den Jód- und Schildrüsenhormongehalt des Blutserums von Lämmern und Kälbern. In: Anke M. *et al.*: Mengen- und Spurenelemente, Arbeitstagung 1987. Teil 2. Karl-Marx-Universität, Leipzig. 381–390.

Groppel B. (1991): Jodmangel beim Tier. In: Anke M., Gürtler H.: Mineralstoffe und Spurelemente in der Ernährung. Verlag Media Touristik, Gersdorf. 127–156.

Herzig I., Suchý P., Kursa J., Krátká V., Písaříková B. (1999a): Krmné produkty řepky olejné a zdravotní stav skotu. In: Sbor. Aktuální otázky výživy a krmení skotu. Výzkumný ústav výživy zvířat s. r. o., Pohořelice. 53–61.

Herzig I., Písaříková B., Kursa J., Říha J. (1999b): Defined iodine intake and changes of its concentration in urine and milk of dairy cows. Vet. Med. – Czech., *44*, 35–40.

Jagoš P., Bouda J. (1981): Základní biochemické a hematologické hodnoty u domácích zvířat a nové způsoby vyjadřování výsledků laboratorních vyšetření. SVS – Oddělení veterinární osvěty, Pardubice. 29 pp.

Janssen K., van der Heide D., Visser T.J., Kaptein E., Beynen A.C. (1994): Thyroid function and deiodinase activities in rats with marginal iodine deficiency. Biol. Trace Elem. Res., *40*, 237–246.

Jenkins K. J., Hidiroglou M. (1990): Effects of elevated iodine in milk replacer on calf performance. J. Dairy Sci., *73*, 804–807.

Köhrle J. (1996): Thyroid hormone deiodinases – a selenoenzyme family acting as gate keepers to thyroid hormone action. Acta Med. Aust., 1/2, 17–30.

Kreze A., Langer P., Klimeš I., Lichardus B. (1993): Praktická endokrinológia (Klinika a patofyziológia endokrinných ochorení). Slovak Academic Press, Bratislava. 549 pp.

Kursa J., Trávníček J., Rambeck W.A., Kroupová V., Vítovec J. (2000): Goitrogenic effects of extracted rapeseed meal and nitrates in sheep and their progeny. Vet. Med. – Czech., *45*, 129–140.

Kvíčala J., Zamrazil V., Bílek R., Soutorová M., Dvořáková M., Šimečková A., Kantorová I., Pobišová Z., Čeřovská J., Jiránek V. (1997): Low selenium status of inhabitants of South Bohemia and its relation to iodine and thyroid hormone metabolism. Biomark. Environ., *1*, 12–20.

Maas J. (1987): Relationship between nutrition and reproduction in beef cattle. Vet. Clin. North Am., *3*, 633–637.

Matoušková O., Chalupa J., Cígler M., Hruška K. (1992): Statistický a grafický systém STAT Plus verze 1.01. Uživatelská příručka. Výzkumný ústav veterinárního lékařství, Brno. 168 pp.

McCoy M.A., Smyth J.A., Ellis W.A., Arthur J.R., Kennedy D.G. (1997): Experimental reproduction of iodine deficiency in cattle. Veter. Rec., *141*, 554–547.

Pajtáš M. (1999): Využitie repky olejnej vo výžive dojníc. In: Sbor. Aktuální otázky výživy a krmení skotu. Výzkumný ústav výživy zvířat s. r. o., Pohořelice. 35–42.

Pandav C.S., Rao A.R. (1997): Iodine Deficiency Disorders in Livestock Ecology and Economics. Oxford University Press, New York. 288 pp.

Písaříková B., Herzig I., Říha J. (1996): Inorganic anion with potential strumigenic effects in potable water for humans and animals. Vet. Med. – Czech, *41*, 1–7.

Rao P.S., Lakshmy R. (1995): Role of goitrogens in iodine deficiency disorders and brain development. Indian J. Med. Res., *102*, 223–226.

Reeds P.J. (1987): Metabolic control and future opportunities for growth regulation. Anim. Prod., *45*, 321–326.

Richter W., Werner E., Bähr H. (1983): Zdraví zvířat. 1. vyd. SZN, Praha. 200 pp.

Rogers P.A.M. (1992): Iodine deficiency in cattle. Irish Vet. News, September 1992. 14–17.

Rudert C.P., Oliver J. (1976): The effect of thiocyanate on the occurrence of goitre in new-born lambs. Rhodes. J. Agric. Res., *14*, 67–72.

Schöne F., Lüdke H.,. Geinitz D., Grün M. (1991): Effect of low or high glucosinolate rapeseed meal on growth, thyroid hormone, vitamin A and trace element status of pigs. In: Proc. 8th International Rapeseed Congress. Saskatoon, *5*, 1577–1584.

Schöne F. (1993): Prüfung von Raps mit unterschiedlichem Glucosinolatgehalt an wachsenden Schweinen – ein Beitrag zur Bewertung nativer Schadstoffe der Nährung. Dtsch Tierarztl. Woch., *100*, 97–99.

Stowe H.D., Herdt T.H. (1992): Clinical asessment of selenium status of livestock. J. Anim. Sci., *70*, 3928–3933.

Suchý P., Herzig I., Krása A., Říha J. (1998): Vliv zkrmování řepkových pokrutin na metabolismus jodu u dojnic. In: III. Dni výživy a veterinárnej dietetiky 17.–18. Septembra 1998. Univerzita veterinárskeho lekárstva, Košice. 161–166,

Šimek M., Šustala M., Vrzalová D., Prokop V., Herzig I., Suchý P., Zeman L., Zemanová D. (1999): Verification of feed products from rapeseed in the assortment of production feed mixtures for dairy cows (in Czech). Metod. Zeměd. Prax. Praha, Ústav zemědělských a potravinářských informací. 26 s. ISBN 80-7271-031-1.

Trávníček J., Kroupová V., Kratochvíl P., Krabačová I. (1999): The effect of superfluous iodine and strumigenic factors on histometric parameters of thyroid gland in layers. Vet. Med. – Czech, *44*, 177–182.

Velíšek J., Hrnčiřík K. (1996): Glukosinoláty v zelenině: jejich nežádoucí a prospěšné účinky. Výživa a potraviny, *51*, 109–110.

Zukalová H., Vašák J. (1978): Gas-chromatographic determination of glucosinolates in the genus *Brassica* (L.) by the trimethylsilyl derivate method (in Czech). Rostl. Výr., *24*, 1009–1017.

Received: 00–01–25
Accepted after corrections: 00–11–10

Corresponding Author:

Ing. Jan Trávníček, CSc., Jihočeská univerzita v Českých Budějovicích, Zemědělská fakulta, Studentská 13, 370 05 České Budějovice, Česká republika
Tel. +420 38 777 26 11, fax +420 38 777 26 21, e-mail: travnic@zf.jcu.cz

Changes in protein content and correlations between contents of amino acids of goat's colostrum during the first 72 hours after parturition

Změny obsahu dusíkatých látek a vzájemných vztahů mezi obsahy aminokyselin mleziva koz v průběhu prvních 72 hodin po porodu

S. GAJDŮŠEK, S. KRÁČMAR, P. JELÍNEK, J. KUCHTÍK

Mendel University of Agriculture and Forestry, Brno, Czech Republic

ABSTRACT: In five female goats of the Czech Brown Polled breed on the 2nd and following lactations, changes were examined in dry matter and basic protein content as well as changes in correlation coefficients between the contents of amino acids found in colostrum from 2 to 72 hours after parturition. The most marked changes in average contents of total dry matter and proteins in colostrum could be observed between the first and the third sampling (i. e. 2, 12 and 24 hours after parturition). The content of total dry matter decreased from 23.8% to 17.5%, that of total nitrogen (TN) dropped from 2.12% to 0.84% and the levels of protein nitrogen (PN) and whey protein nitrogen (WPN) decreased from 1.92% to 0.80% and from 1.40% to 0.38%, respectively. On the other hand, casein nitrogen (CN) increased from 0.15% to 0.42% and the casein number rose from 8.0% to 52.2%. The CN content showed the highest variability. In the next period (i. e. between 36 and 72 hours after parturition), changes in all components studied were much smaller. The content of TN dropped from 0.90% to 0.84%, that of PN from 0.85% to 0.80% and that of WPN from 0.36% to 0.25%. In this period, further increase of the CN content from 0.49% to 0.56% occurred, and the casein number rose from 57.8% to 69.4%. Between the 1st and the 2nd sampling, the content of non-protein nitrogen (NPN) in colostrum was approximately 9% of TN and thereafter, i. e. 24 hours after parturition, it varied between 4.7 and 4.8%. Two, 12 and 24 hours after parturition, urea nitrogen (UN) represented 9%; 23% and 52.3 % of NPN. In the next period, the percentage of UN in NPN decreased slightly from 32.5 to 30%. Changes of amino acid (AA) contents in the colostrum during the first 72 hours after parturition were published in our earlier publication (Kráčmar *et al.*, 1999). In spite of a high variability of AA contents in colostrum sampled in 2, 12 and 48 hours after parturition, the values of all correlation coefficients of AA contents in individual colostrum samples were found to be very high or high, and all positive (except His and, after 12 hours, also Phe).

Keywords: goats; colostrum; proteins; correlation of AA aminokyselinami

ABSTRAKT: U pěti koz plemene hnědá krátkosrstá na druhé a vyšší laktaci byly sledovány změny obsahu sušiny a základních dusíkatých látek (celkový, proteinový, bílkovin mléčného séra, kazeinu a močoviny) a změny hodnot korelačních koeficientů mezi obsahy aminokyselin mleziva od 2 do 72 h po porodu. Nejmarkantnější změny v průměrných hodnotách celkové sušiny a dusíkatých látek kolostra jsou pozorovatelné mezi prvním a třetím odběrem vzorků (2, 12 a 24 h po porodu). Celková sušina klesá z 23,8 na 17,5 %, celkový dusík (TN) klesá z 2,12 na 0,84 %, proteinový dusík (PN) z 1,92 na 0,80 % a dusík syrovátkových bílkovin (WPN) z 1,40 na 0,38 %. Naopak kazeinový dusík (CN) se zvyšuje z 0,15 na 0,42 % a kazeinové číslo stoupá z 8,0 na 52,2 %. Obsah CN vykazuje největší variabilitu. V dalším období (36 až 72 h po porodu) jsou již změny všech sledovaných složek mnohem menší. Obsah TN klesl z 0,90 na 0,84 %, obsah PN z 0,85 na 0,80 % a obsah WPN z 0,36 na 0,25 %. V tomto období došlo k dalšímu zvyšování obsahu CN z 0,49 na 0,56 % a kazeinové číslo se zvýšilo z 57,8 až na 69,4 %. Obsah nebílkovinného dusíku (NPN) činil v mlezivu z 1. a 2. odběru vzorku asi 9 % z TN, od 24 h po porodu kolísal mezi 4,7 a 4,8 %. Z NPN činil 2 h po porodu podíl močovinového dusíku 9 %, po 12 h kolem 23 % a maximum 24 h po porodu (52,3 %). V dalším období se procentuální

Supported by the Research Project CEZ:J08/98:432100001.

podíl UN z NPN nepatrně snižuje z 32,5 na 30 %. Změny obsahu aminokyselinového (AA) složení mleziva v prvních 72 h po porodu byly uvedeny v předcházející publikaci (Kráčmar *et al.*, 1999). I přes zjištěnou vysokou variabilitu obsahu jednotlivých AA v kolostru, odebraném 2, 12 a 48 h po porodu, byly v těchto obdobích zjištěny hodnoty všech korelačních koeficientů (kromě His a po 12 h i Phe) mezi obsahy jednotlivých AA v individuálních vzorcích kolostra velmi vysoké, resp. vysoké, a všechny pozitivní.

Klíčová slova: koza; mlezivo; dusíkaté látky; korelace mezi aminokyselinami

INTRODUCTION

Colostrum, as the first secretion of the mammary gland after parturition, differs significantly from "ripened" milk (Khalil *et al.*, 1992). In the first hours after parturition, colostrum is rich in proteins. The highest proportion of these substances is represented by serum proteins, first of all immunoglobulins, both in cow's colostrum (Klimeš *et al.*, 1986) and goat's colostrum (Hadjipanayitou, 1995); however, colostrum of all mammalian species contains also high amounts of volatile peptides and amino acids (Schablin,1987; Saito *et al.*, 1993) as well as increased concentrations of lactoferrin (Abd-El-Gawad *et al.*, 1986, 1996) and many other enzymes. That is why the spectrum of amino acids in colostrum is not dependent only on the concentration of serum proteins and casein. Contents of all these components influence correlations of amino acid present in colostrum.

The objective of this paper, which is a continuation of our earlier study about changes in amino acid composition of goat colostrum (Kráčmar *et al.*, 1999), is to assess changes in the contents of essential nitrogen compounds in goat's colostrum in the first 72 hours after parturition and to evaluate changes of correlation coefficients for the amino acid composition.

MATERIAL AND METHODS

Composition of goat's colostrum was studied in 5 dairy goats of the Czech Brown Polled breed on the 2nd and subsequent lactations from 2 to 72 hours after parturition within the period February–March 1998.

Goats received feeding rations consisting of:
- maize silage, feeding ration of 3.0 kg
- fodder beet, feeding ration of 2.0 kg
- bulk fodder, feeding ration of 0.5 kg
- meadow hay, fed *ad libitum*
- oat straw with undersown grass, fed *ad libitum*

Colostrum samples were taken 2, 12, 24, 36, 48 and 72 hours after parturition; colostrum was homogenised in the batches of ca. 100–300 ml, and the homogenised samples were thereafter frozen.

Total nitrogen was determined according to Kjeldahl using Kjeltec Auto 1031 Analyser (manufactured by Tecator). Total proteins, serum proteins and casein were determined using the apparatus Pro Milk II (manufactured by Foss Electric). Urea content was determined spectrophotometrically using *p*-dimethylaminobenzaldehyde. All substances were expressed as percentages of total nitrogen (calculated with factor 6.37). Amino acids were determined chromatographically and results were published by Kráčmar *et al.* (1999). All results were evaluated using the variation statistics (ANOVA) and correlation analysis according to Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Changes in colostrum dry matter and protein contents observed in the first 72 hours after parturition are presented in Tab. 1. Colostrum produced by the mammary gland within the first 2 hours after parturition shows a high content of dry matter. This dry matter consists of more than 56% of crude proteins (the factor 6.37 is used for conversion of nitrogen to proteins). More than 90% of true protein nitrogen corresponds to protein nitrogen. Of the proteins investigated, the highest variability was observed in the contents of casein and urea nitrogen (35.2% and 46.3%, respectively). The casein number was only 8%.

In the course of lactation, the content of all components examined decreased markedly with the exception of casein; the proportion of proteins in the total dry matter decreased as well. More marked were the changes observed after the first, second, and third milking (2, 12 and 24 hours after parturition). Twelve hours after parturition, the crude protein content was only 40% of dry matter (the factor 6.37 is used for conversion of nitrogen to proteins); however, the percentage of protein nitrogen in total nitrogen was always higher than 90%. However, the proportion of serum proteins in protein nitrogen decreased while in the casein number it increased to approximately 49%. The contents of casein showed the highest variability. Similar results were presented also by Singh *et al.* (1972), who found higher initial values of casein.

In the next period, the decrease of the total nitrogen and protein nitrogen contents was relatively low; the percentage of protein nitrogen dropped to ca 95% and practically did not change by the end of the period under study. In colostrum sampled 24 hours after parturition, the contents of total nitrogen, protein nitrogen and serum protein nitrogen decreased. A slight decrease in casein nitrogen was also registered, although always with a high variation of the coefficient value and, finally, the casein

number rose to ca 52%. In samples obtained 48 hours after parturition, a slight temporary increase of dry matter, total nitrogen and pure protein nitrogen was observed and the casein number rose to ca 66%. In colostrum sampled 72 hours after parturition, the casein number was already over 69%. The content of urea nitrogen after parturition was low (0.019%) but with a high variability. In samplings carried out 12 and 24 hours after parturition, a temporary increase was registered. The percentage of urea nitrogen in non-protein nitrogen reached its maximum (52.3%) 24 hours after parturition and in the next period (i. e. 72 hours after parturition) it dropped to 30%.

The variability of amino acid composition in the colostrum and its changes within the first 72 hours after parturition were described in our previous study (Kráčmar *et al.*, 1999). It was found that the contents of all amino acids showed the highest variability 2 hours after parturition. Besides the animal's individuality the cause of this variability may be the amount and production rate of the colostrum. In spite of that, serum proteins showed the highest percentage in colostrum proteins after parturition; this concerned above all immunoglobulins, the content of which decreased markedly during the next days (Quilies *et al.*, 1991, 1992). High contents of other components of peptidic nature could also be expected in the colostrum, especially of volatile peptides, amino acids, enzymes, etc. (Schablin, 1987; Saito *et al.*, 1993). In spite of a high variability in the final pattern of AA in colostrum and their individual levels, very high and highly positive correlation coefficients were found out (Tab. 2). Only the histidine content did not indicate any significant correlation with the contents of other AA. Twelve hours after parturition, in spite of the still high value of variation coefficients of all AA, the values of all correlation coefficients, except for His, were again very high and high, and they were again positive in all cases (Tab.3).

Table 1. Changes of dry matter and basic protein of goat's colostrum in the first 72 hours after parturition (%)

	2			12			24		
	Mean	S_E	v%	Mean	S_E	v%	Mean	S_E	v%
Solids	23.83	± 1.063	10.0	17.61	± 1.127	14.3	17.53	± 0.191	2.4
Total N	2.115	± 0.340	16.3	1.116	± 0.203	18.1	0.836	± 0.051	6.3
Protein N	1.922	± 0.310	16.3	1.015	± 0.184	18.1	0.796	± 0.050	6.3
Serum protein N	1.402	± 0.071	5.2	0.518	± 0.289	55.9	0.380	± 0.071	17.3
Casein N	0.154	± 0.070	46.3	0.497	± 0.200	40.3	0.416	± 0.110	25.7
Urea N	0.019	± 0.012	35.2	0.023	± 0.005	22.7	0.021	± 0.003	17.1
	36			48			72		
	Mean	S_E	v%	Mean	S_E	v%	Mean	S_E	v%
Solids	16.53	± 1.046	14.1	18.29	± 0.794	9.7	16.03	± 0.617	8.6
Total N	0.896	± 0.052	6.1	0.868	± 0.082	9.7	0.844	± 0.072	8.5
Protein N	0.853	± 0.050	6.1	0.827	± 0.081	9.7	0.804	± 0.071	8.5
Serum protein N	0.360	± 0.062	17.8	0.284	± 0.031	9.7	0.246	± 0.032	12.5
Casein N	0.493	± 0.040	9.0	0.543	± 0.051	9.6	0.558	± 0.053	8.6
Urea N	0.014	± 0.003	18.0	0.014	± 0.005	16.8	0.012	± 0.002	9.9

Table 2. Values of correlation coefficients for AAs of goat's colostrum 2 hours after parturition

	Asp	Met	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ile	Leu	Tyr	Phe	His	Lys	Arg
Cys	0.935	0.971	0.955	0.956	0.913	0.969	0.937	0.888	0.955	0.913	0.899	0.903	0.896	0.152	0.952	0.984
Asp	–	0.961	0.994	0.997	0.993	0.993	0.906	0.985	0.988	0.993	0.987	0.949	0.981	0.031	0.997	0.981
Met		–	0.986	0.966	0.963	0.981	0.860	0.953	0.969	0.960	0.961	0.973	0.918	0.263	0.979	0.978
Thr			–	0.993	0.991	0.997	0.891	0.983	0.991	0.988	0.988	0.968	0.967	0.125	0.999	0.988
Ser				–	0.985	0.997	0.930	0.972	0.991	0.985	0.976	0.940	0.977	0.027	0.997	0.991
Glu					–	0.981	0.853	0.998	0.973	0.999	0.997	0.973	0.964	0.113	0.993	0.962
Pro						–	0.922	0.969	0.995	0.980	0.976	0.949	0.971	0.008	0.997	0.996
Gly							–	0.821	0.920	0.859	0.829	0.769	0.905	–0.193	0.901	0.944
Ala								–	0.961	0.996	0.998	0.975	0.956	0.131	0.984	0.944
Val									–	0.967	0.971	0.926	0.985	0.024	0.990	0.991
Ile										–	0.993	0.973	0.957	0.112	0.992	0.960
Leu											–	0.972	0.965	0.128	0.988	0.953
Tyr												–	0.886	0.338	0.962	0.928
Phe													–	–0.109	0.972	0.960
His														–	0.009	0.006
Lys															–	0.986

Table 3. Values of correlation coefficients for AAs of goat's colostrum 12 hours after parturition

	Asp	Met	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ile	Leu	Tyr	Phe	His	Lys	Arg
Cys	0.770	0.841	0.754	0.869	0.730	0.838	0.829	0.862	0.778	0.849	0.807	0.788	0.449	–0.212	0.803	0.623
Asp	–	0.913	0.929	0.954	0.992	0.977	0.994	0.979	0.997	0.981	0.993	0.415	0.899	0.196	0.997	0.946
Met		–	0.862	0.855	0.923	0.961	0.914	0.906	0.894	0.921	0.912	0.572	0.691	–0.185	0.919	0.753
Thr			–	0.857	0.889	0.872	0.928	0.888	0.949	0.875	0.961	0.623	0.751	0.002	0.951	0.803
Ser				–	0.929	0.954	0.978	0.993	0.957	0.985	0.955	0.472	0.820	0.235	0.957	0.926
Glu					–	0.981	0.977	0.963	0.978	0.974	0.971	0.339	0.914	0.197	0.980	0.942
Pro						–	0.980	0.981	0.962	0.991	0.965	0.453	0.831	0.008	0.937	0.898
Gly							–	0.994	0.995	0.991	0.995	0.480	0.862	0.169	0.996	0.934
Ala								–	0.977	0.997	0.977	0.477	0.840	0.179	0.981	0.929
Val									–	0.974	0.998	0.452	0.887	0.201	0.998	0.941
Ile										–	0.973	0.447	0.849	0.169	0.978	0.930
Leu											–	0.509	0.855	0.137	0.999	0.917
Tyr												–	–0.008	–0.613	0.486	0.156
Phe													–	0.522	0.865	0.972
His														–	0.144	0.499
Lys															–	0.924

Table 4. Values of correlation coefficients for AAs of goat's colostrum 24 hours after parturition

	Asp	Met	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ile	Leu	Tyr	Phe	His	Lys	Arg
Cys	0.898	0.003	0.639	0.762	0.650	0.178	0.536	–0.831	0.205	0.467	0.001	0.210	–0.684	–0.908	–0.006	–0.003
Asp	–	–0.371	0.265	0.426	0.854	–0.201	0.407	–0.690	–0.190	0.802	–0.362	0.594	–0.814	–0.754	–0.330	–0.334
Met		–	0.558	0.473	–0.713	0.563	–0.119	–0.358	0.621	–0.730	0.590	–0.726	0.611	–0.315	0.527	0.365
Thr			–	0.985	0.121	0.861	0.715	–0.457	0.881	–0.361	0.770	–0.614	–0.292	–0.559	0.450	0.688
Ser				–	0.252	0.765	0.728	–0.568	0.788	–0.197	0.652	–0.468	–0.404	–0.671	0.367	0.574
Glu					–	–0.150	0.593	–0.219	–0.183	0.772	–0.282	0.563	–0.979	–0.328	–0.377	–0.125
Pro						–	0.692	0.003	0.997	–0.715	0.986	–0.894	–0.005	–0.006	0.592	0.950
Gly							–	–0.004	0.638	–0.001	0.574	–0.293	–0.734	–0.203	0.352	0.665
Ala								–	–0.029	–0.356	0.158	–0.225	0.201	0.986	0.002	0.326
Val									–	–0.721	0.980	–0.898	–0.001	–0.122	0.596	0.926
Ile										–	–0.815	0.952	–0.630	–0.360	–0.580	–0.728
Leu											–	–0.950	0.008	0.007	0.610	0.965
Tyr												–	–0.385	–0.185	–0.611	–0.876
Phe													–	0.333	0.270	–0.007
His														–	0.002	0.216
Lys															–	0.496

Table 5. Values of correlation coefficients for AAs of goat's colostrum 36 hours after parturition

	Asp	Met	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ile	Leu	Tyr	Phe	His	Lys	Arg
Cys	0.329	–0.714	0.003	0.612	0.576	0.489	0.433	0.702	0.714	0.795	0.605	0.207	0.664	0.443	0.622	0.196
Asp	–	0.362	0.491	0.603	0.570	0.880	–0.539	0.885	0.600	0.476	0.575	0.135	–0.209	0.258	0.362	0.262
Met		–	0.531	0.001	0.003	0.255	–0.604	0.005	–0.109	–0.262	0.007	–0.233	0.890	0.003	–0.137	0.002
Thr			–	0.811	0.836	0.766	0.009	0.456	0.723	0.623	0.816	0.002	–0.395	0.806	0.763	0.543
Ser				–	0.998	0.903	0.299	0.788	0.990	0.954	0.999	0.152	0.007	0.885	0.961	0.550
Glu					–	0.886	0.320	0.753	0.982	0.944	0.999	0.158	0.005	0.899	0.969	0.573
Pro						–	–0.110	0.901	0.887	0.801	0.889	0.008	–0.147	0.681	0.749	0.413
Gly							–	–0.170	0.326	0.479	0.330	–0.136	0.409	0.585	0.530	0.005
Ala								–	0.827	0.758	0.764	0.327	0.209	0.437	0.632	0.442
Val									–	0.979	0.988	0.186	0.186	0.851	0.955	0.532
Ile										–	0.956	0.009	0.264	0.871	0.953	0.423
Leu											–	0.139	0.007	0.901	0.968	0.546
Tyr												–	0.651	–0.176	0.183	0.842
Phe													–	–0.009	0.204	0.380
His														–	0.926	0.319
Lys															–	0.587

The value of correlation coefficients decreased in the case of Phe. Twenty-four hours after parturition (Tab. 4), most values of correlation coefficients markedly decreased and negative correlations between several contents of AA were found out. Individual levels of AA showed the lowest values of coefficients of variation, similarly like in our previous study. As compared with the values calculated for colostrum produced 12 and 24 hours after parturition a decrease in the values of correlation coefficients (for Ser, Cys, Thr, Lys and Arg) occurred also 36 hours after parturition while for the others the calculated values rose up again (Tab. 5). Forty-eight hours after parturition, coefficients of variation rose significantly for all AA (with the maximum for Phe and His – 50.18% and 80.8%, resp.); the values of correlation coefficients were again very high and high, and all were positive (Tab. 6). Only in the case of His and Phe, the majority and/or a part of measured values, respectively, were only high. Seventy-two hours after parturition (Tab. 7), the values of coefficients of variation decreased for the majority of AA and the values of correlation coefficients also markedly decreased in the majority of cases; the content of Phe did not indicate any significant correlation with any other AA.

Taking into account the broad spectrum of substances with peptidic character and different levels of AA that may occur in the colostrum it is possible to assume a high variability in the contents of all AA (depending on the animal's individuality). This variability was confirmed in our previous study (Kráčmar *et al.*, 1999). But in spite of the relatively low number of analysed colostrum samples ($n = 5$), it is of interest that in the period of an increased variability in their contents (2, 12 and 48 hours after parturition), the values of correlation coefficients calculated for individual AA were high or very high in the majority

Table 6. Values of correlation coefficients for AAs of goat's colostrum 48 hours after parturition

	Asp	Met	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ile	Leu	Tyr	Phe	His	Lys	Arg
Cys	0.990	0.890	0.996	0.995	0.997	0.998	0.961	0.994	0.996	0.996	0.996	0.970	0.917	0.856	0.996	0.997
Asp	–	0.937	0.996	0.996	0.993	0.994	0.998	0.992	0.997	0.989	0.994	0.966	0.885	0.812	0.998	0.987
Met		–	0.905	0.930	0.891	0.915	0.952	0.890	0.915	0.890	0.898	0.863	0.706	0.724	0.916	0.879
Thr			–	0.995	0.999	0.996	0.973	0.999	0.999	0.997	0.999	0.978	0.919	0.838	0.998	0.997
Ser				–	0.993	0.999	0.978	0.988	0.997	0.991	0.992	0.959	0.881	0.836	0.998	0.988
Glu					–	0.995	0.967	0.999	0.998	0.997	0.999	0.977	0.926	0.840	0.998	0.998
Pro						–	0.971	0.991	0.997	0.994	0.994	0.964	0.896	0.848	0.998	0.993
Gly							–	0.964	0.979	0.954	0.968	0.921	0.816	0.718	0.981	0.953
Ala								–	0.996	0.997	0.999	0.985	0.937	0.844	0.994	0.998
Val									–	0.995	0.998	0.971	0.905	0.827	0.999	0.994
Ile										–	0.998	0.986	0.937	0.876	0.993	0.999
Leu											–	0.982	0.929	0.845	0.997	0.998
Tyr												–	0.967	0.893	0.967	0.985
Phe													–	0.884	0.899	0.942
His														–	0.823	0.869
Lys															–	0.993

Table 7. Values of correlation coefficients for AAs of goat's colostrum 72 hours after parturition

	Asp	Met	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ile	Leu	Tyr	Phe	His	Lys	Arg
Cys	0.815	0.736	0.824	0.786	0.940	0.916	0.950	0.878	0.914	0.938	0.952	0.907	0.004*	0.995	0.938	0.995
Asp	–	0.958	0.942	0.655	0.811	0.958	0.933	0.791	0.941	0.890	0.903	0.635	–0.487	0.840	0.922	0.845
Met		–	0.863	0.575	0.685	0.867	0.855	0.650	0.821	0.824	0.793	0.564	–0.434	0.757	0.802	0.753
Thr			–	0.850	0.756	0.974	0.958	0.709	0.933	0.793	0.864	0.546	–0.342	0.820	0.945	0.872
Ser				–	0.592	0.807	0.842	0.488	0.732	0.577	0.678	0.474	0.161	0.732	0.800	0.824
Glu					–	0.883	0.888	0.988	0.934	0.970	0.981	0.933	–0.170	0.966	0.925	0.936
Pro						–	0.993	0.844	0.986	0.907	0.956	0.717	–0.298	0.921	0.990	0.946
Gly							–	0.834	0.971	0.913	0.954	0.758	–0.185	0.948	0.986	0.973
Ala								–	0.915	0.951	0.961	0.904	–0.275	0.918	0.891	0.876
Val									–	0.935	0.982	0.767	–0.345	0.932	0.994	0.941
Ile										–	0.978	0.915	–0.229	0.967	0.920	0.930
Leu											–	0.872	–0.250	0.973	0.975	0.961
Tyr												–	0.105	0.925	0.769	0.868
Phe													–	–0.034	–0.254	–0.001
His														–	0.944	0.989
Lys															–	0.964

of cases, and all were positive. Therefore an increase in the content of one amino acid was accompanied by an increase in the contents of all other amino acids.

REFFERENCES

Abd-El-Gawad I.A., Mahfouz M.B., ALI A.A., Hewedi M. (1986): The electrophoretic pattern of goats milk protein during various periods of lactation. Ann. Agric. Sci., Moshtohor, *24*, 1469–1475.

Abd-El-Gawad I.A., El-Sayed E.M., Mahfouz M.B., Abd-El-Salam A.M. (1996): Changes of lactoferrin concentration in colostrum and milk from different species. Egyptian J. Dairy Sci., *24*, 297–308.

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

Khalil H.M., Fekry M.M., Al-Ashmawy A.M., Kheir A.A. (1992): Analytical study of protein of milk products. Egypt. J. Pharmac. Sci., *33*, 341–355.

Klimeš J., Jagoš P., Bouda J., Gajdůšek S. (1986): Basic qualitative parameters of cow colostrum and their dependence on season and post partum time. Acta Vet. Brno, *55*, 23–39.

Kráčmar S., Gajdůšek S., Jelínek P., Zeman L., Kozel V., Kozlová M., Kráčmarová E. (1999): Changes in amino acid composition of goat's colostrum during the first 72hours after parturition. Czech J. Anim. Sci., *44*, 541–545.

Quiles A.J., Gonzalo C., Fuentes F., Hevia M., Sanchez J.M. (1991): Protein composition and variation of caprine colostrum (Muriano-Granadina breed) by means of polyacrylamide-SDS gel electrophoresis. Anim. Product., *52*, 311–316.

Quiles A., Hevia M., Fuentes F., Barcina Y., Ramirez A. (1992): Electrophoretic fractions of proteins of milk from Murciano-Granadina goat during lactation. Anal. Vet. de Murcia, *8*, 7–13.

Saito T., Yoshida T., Itoch T. (1993): Composition of free form glycopeptides isolated from bovine colostrum. Int. Dairy J., *3*, 129–139.

Schablin E. (1987): Untersuchungen zum Stickstoff- and Aminosäurengehalt der Kolostralmilch verschiedener Haustire und der Kolostralmilch und reifen Milch von Frauen. [PhD Dissertation.] Giesen, Just-Liebig-Universität.

Singh N.P., Sachdeva K.K., Sengar O.P.S. (1972): A study on the nitrogen distribution in goat's milk. Milchwissenschaft, *23*, 165–167.

Snedecor G.W., Cochran W.G. (1967): Statistical Methods. Iowa State Univ. Press. 534 pp.

Received: 99–11–02
Accepted after corrections: 00–11–10

Corresponding Author:

Prof. Ing. Stanislav Gajdůšek, DrSc., Mendelova zemědělská a lesnická univerzita, Zemědělská 1, 613 00 Brno, Česká republika
Tel. +420 5 45 13 31 93, fax +420 5 45 22 20 44, e-mail: gajd@mendelu.cz

Milk and blood as alternative sources of mRNA for studies of genetic polymorphism of alpha S1 casein in goats

Mléko a krev jako alternativní zdroje pro studium genetického polymorfismu alfa S1 kazeinu u koz

M. Tokarska[1], D. Szumska[1] B. Kosowska[1], T. Dobosz[2], Z. Zdrojewicz[3]

[1]*Department of Genetics and Animal Breeding, Agricultural University, Wrocław, Poland*
[2]*Department and Institute of Forensic Medicine, Medical Academy, Wrocław, Poland*
[3]*Department and Clinic of Endocrinology and Diabetology, Medical University, Wrocław, Poland*

ABSTRACT: Caprine αS1 casein gene polymorphism is responsible for quality of main processing parameters of milk, related with casein and protein content, processing quality and cheese flavour. The main 7 alleles are divided into 4 groups: strong alleles (which code 3.6 g/l), medium allele (1.6 g/l), weak alleles (0.6 g/l) and null allele, associated with lack of αS1 casein in milk. Milk mononuclear cells (MNC) and peripheral blood mononuclear cells (PBMC) of 16 goats were used as a source of mRNA of αS1 casein gene. Complementary DNA (cDNA) was obtained from both, milk and blood sources using RT-PCR. Specific primers allowed to distinguish strong alleles (*A, B, C*) from weak alleles (*D, E*) and medium allele (*E*), since allele *F* has 111 bp deletion (exons 9–11), allele *D* deletion of 33 bp (exon 9) and allele *E* is associated with 457 bp insertion in exon 19. Identical results obtained using both, somatic cells of milk and peripheral blood leukocytes indicate that they may be used alternatively as mRNA sources for genetic polymorphism analyses of αS1 casein gene in goats. The disadvantage was a failure to isolate mRNA from either milk or blood collected in June–October.

Keywords: polymorphism; αS1 casein; cDNA; goats; PCR.

ABSTRAKT: Genetický polymorfismus αS1 kazeinu u koz určuje kvalitu hlavních zpracovatelských parametrů mléka v souvislosti s obsahem kazeinu a bílkovin, zpracovatelskou kvalitu a chuť sýra. Hlavních sedm alel se rozděluje do čtyř skupin: silné alely (které kódují 3,6 g/l), středně silná alela (1,6 g/l), slabé alely (0,6 g/l) a nulová alela související s absencí αS1 kazeinu v mléce. Jako zdroj mRNK genu αS1 kazeinu jsme použili mononukleární buňky mléka (MNC) a mononukleární buňky periferní krve (PBMC) od 16 koz. Komplementární DNK (cDNA) jsme získali z mléka i z krve za použití metody RT-PCR. Specifické primery umožnily rozlišit silné alely (*A, B, C*) od slabých alel (*D, E*) a středně silné alely (*E*), protože alela *F* vykazuje deleci 111 bp (exony 9–11), alela *D* deleci 33 bp (exon 9) a alela *E* je spojovaná s inzercí 457 bp v exonu 19. Shodné výsledky, které jsme získali při použití jak somatických buněk mléka, tak leukocytů periferní krve naznačují, že je lze používat alternativně jako zdroje mRNK k analýzám genetického polymorfismu genu αS1 kazeinu u koz. Nevýhodou bylo to, že se nepodařilo mRNK izolovat z mléka ani z krve, které byly odebrány v období červen–říjen.

Klíčová slova: polymorfismus; αS1 kazein; cDNK; kozy; PCR

INTRODUCTION

It is already well known that genetic polymorphism of caprine αS1 casein gene determines casein and overall protein content, fat content, curd strength and cheese flavour. The main seven αS1 casein alleles determine different expression levels, thus they are assigned to 4 groups. Alleles (*A, B, C*), called "strong" determine high αS1 casein content in milk (3.6 g per litre); allele *E* – "medium" is associated with 1.6 of casein per litre; alleles "weak" – *D, F* – code 0.6 g/l; allele *0* is responsible for lack of αS1 casein in milk.

Reported molecular analyses and genotyping of caprine αS1 casein gene in milking goats have been performed mostly by milk-isolated proteins electrophoresis (Grosclaude *et al.*, 1987). Buck genotyping is based on

DNA isolated from peripheral blood leukocytes using Restriction Fragment Length Polymorphism (RFLP) method (Leroux *et al.*, 1990). Some analyses of the structure and sequence of the gene were performed on mRNA isolated from mammary gland cells, collected both, *intra vitam* and *post mortem*, using a biopsy method (Leroux *et al.*, 1992; Pérez *et al.*, 1994). Biopsy is an invasive and anaesthetic usage requiring method; quarantine time can be an additional problem in genotyping milking goats and when performed *post mortem*, it has obviously no selection meaning.

Analysing the polymorphism of αS1 casein at mRNA (cDNA) level is easier than at DNA level since differences between strong and weak alleles concern distinct deletions of 33 to 111 bp in αS1 casein mRNA instead of point mutations and presence of long intron sequences in genomic DNA. As reported by Tokarska *et al.* (1999), somatic cells of goat milk are also a source of αS1 casein mRNA. Although collecting milk is easy and non-invasive, genotyping analyses based on milk can be performed only for milking, adult females. Though the transcription of αS1 casein gene in blood cells seems improbable, it seemed important to check whether mRNA can be obtained from blood leukocytes as well.

MATERIAL AND METHODS

Material

Blood and milk samples were collected from 16 goats (Saanen and colour breeds) of previously determined αS1 casein genetic variants (Tokarska *et al.*, 1999) coming from private farms in the Lower Silesia region. Approximately 50 ml of milk and 9 ml of peripheral blood on EDTA were collected.

Methods

Isolation of mRNA. Total RNA from both sources was isolated using the same, Gibco BRL procedure, based on TRIzol Reagent.

Procedure. Samples were pelleted by centrifugation. Cells were lysed with TRIzol by repetitive pipetting and incubated 5 min at room temperature. Then 0.2 ml of chloroform per each 1 ml of TRIzol was added. After 15s shaking samples were incubated at room temperature for 3 min and then centrifuged for 15 min at 3°C (max. 12 000 x g). The upper-water phase was transferred to another tube, and 0.5 ml of isopropylalcohol was added per each 1 ml of TRIzol Reagent used. Samples were incubated for 10 min and centrifuged for 10 min at 5°C (max. 12 000 x g). Supernatant was removed, pellet was washed with 75% ethanol and centrifuged for 5 min (max 7500 x g) at 5°C. RNA pellet was briefly dried (in vacuum-dry) for 10 min. RNA was dissolved in deionised water including 2% DEPC and incubated for 5 min at 60°C.

Reverse transcription. The presence of total RNA in isolated samples was checked using 1.0% agarose electrophoresis. Stratagene RT-PCR kit was used for reverse transcription. The reaction was performed on PTC 100 (MJ Research) cycler.

Obtained cDNA of caprine S1 casein was amplified, using a pair of specific primers: F1/R3, described by Tokarska *et al.* (1999), of the following sequences:

F1: 5' – GAC AAC CAT GAA ACT TCT CAT CC – 3',

R3: 5' – CTT ACA GGA GAG GTG ATT CAA AG – 3'.

Product of F1/R3 amplification covers almost the whole length of the transcript, including deletions in alleles *D*, *F* and *G* and allele *E*-insertion. We performed a computer search for identity of the primer sequences with known sequences in GenBank and it showed no homology with any other sequences of goat genome than αS1 casein gene.

All PCR reactions in this study were performed on PTC 100, MJ Research cycler, using the following conditions: 94°C/120 s, 30x(94°C/60 s, 68°C/30 s, 72°C/45 s), 72°C/420 s (Tokarska *et al.*, 1999).

Agarose electrophoresis. PCR products were analysed by electrophoresis in 1.5% agarose gels (3/4 of Sea Kem® Le Agarose and ¼ of NuSieve®GTG®Agarose) in 0.5% TBE; 180 V for 45 min. Visualisation was carried out using GelDoc 2000 system (BioRad).

RESULTS

It has to be underlined that we failed in attempts to isolate αS1 casein mRNA from either milk or blood collected during summer and autumn months (June–October). Thus we collected samples starting soon after parturition until May.

In this study only strong alleles and weak allele *F* were detected. It might be due to the small and related population of goats used for analyses. After amplifications of αS1 casein cDNA with primers F1 and R3, we expected 1002 bp fragments for strong alleles and 890 bp for allele *F*. Allele *D* (not detected in this study) results in approximately 969 bp band.

Figures 1A and 1B present results of amplification of cDNA isolated from milk somatic cells of goats while Fig. 2 shows results of analogous amplification, but performed on cDNA isolated from blood leukocytes of the same goats.

In all figures, heterozygotes carrying strong allele and allele *F* are described as *S/F*, while strong homozygotes as *S/S*. Strong alleles were not differentiated because each of them codes the same, high, amount of protein.

After amplification, we obtained a single PCR product of 1 002 bp for strong homozygotes *S/S* and two PCR products of 1 002 and 890 bp for heterozygotes *S/F*. Analysis of cDNA obtained from both sources, milk somatic cells and blood gave identical results for all analysed goats, which is shown in Figs. 1A, 1B and 2.

The band sizes (bp) were analysed using QuantityOne ®Quantitation Software (BIO-RAD).

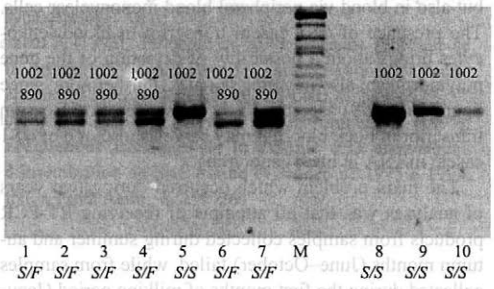

Figure 1A. Agarose electrophoresis of F1/R3 PCR products, performed on cDNA isolated from milk somatic cells of goats. Size marker: Lambda DNA/Eco471 (*Ava* II) Marker 13 (MBI Fermentas)

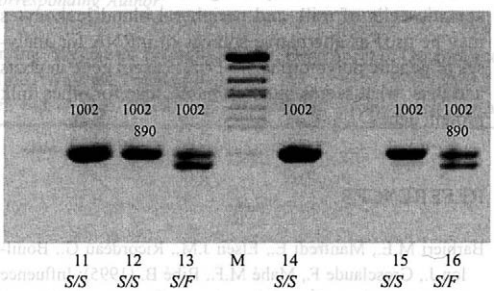

Figure 1B. Agarose electrophoresis of F1/R3 PCR products, performed on cDNA isolated from milk somatic cells of goats

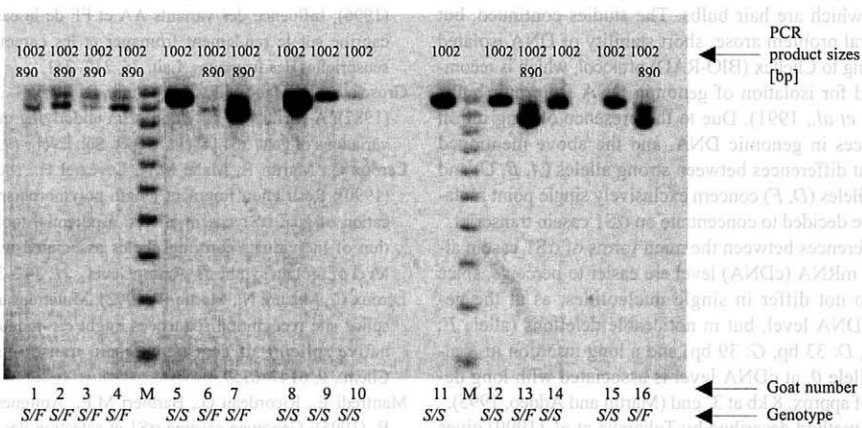

Figure 2. Agarose electrophoresis of F1/R3 PCR products, performed on cDNA isolated from blood somatic leukocytes of the same goats. Size marker: EZ load ™ 100 bp Molecular Ruler (BIO-RAD)

DISCUSSION

As it was stated above, only strong alleles and weak allele *F* were detected in this study. It might be due to the small and related population of goats used for analyses. According to Tokarska *et al.* (1999), additional amplifications should be performed to distinguish medium allele *E*, which seems comparatively hard to detect due to its spacial conformation and thus threefold lower amount of its transcript (Pérez *et al.*, 1994).

Detection of genetic variants of αS1 casein in milk type breeds of goats is much more important than in any other domestic animals, since they determine milk processing parameters and sensory value of goat milk (Barbieri *et al.*, 1995; Remeuf, 1993; Vassal *et al.*, 1994; Delacroix-Buchet *et al.*, 1996).

Different sources of genetic information have been used for genotyping of goat αS1 casein. Usually reported methods of detection of αS1 casein allele variants generally concerned protein analysis (Grosclaude *et al.*, 1987) or RFLP variants based on genomic DNA (Leroux *et al.*, 1990; Manfredi *et al.*, 1995; Ricordeau *et al.*, 1995).

Milk protein electrophoresis method (Grosclaude *et al.*, 1987), followed by visual estimation of the amount of αS1 casein, used for adult females genotyping is the most widely used method for genotyping milking goats. Though it is comparatively easy to perform, but the results may be easily over- or underestimated. Protein electrophoresis method, though easy to perform, thus widely used, may be deceptive (Tokarska *et al.*, 1999). Genotyping of bucks relies on RFLP analysis of blood-isolated genomic DNA. The method requires the use of numerous restriction enzymes necessary for each genotyping (Leroux *et al.*, 1990) whilst it is still impossible to differentiate "weak" allele *D*.

Initially we tried to use an easy-to-collect source of DNA, which are hair bulbs. The studies continued, but a general problem arose: short stability of DNA isolated according to Chellex (BIO-RAD) protocol, which is recommended for isolation of genomic DNA from hair bulbs (Walsh *et al.*, 1991). Due to the presence of long intron sequences in genomic DNA, and the above mentioned fact that differences between strong alleles (*A*, *B*, *C*) and weak alleles (*D*, *F*) concern exclusively single point mutations we decided to concentrate on αS1 casein transcript.

Differences between the main forms of αS1 casein alleles at mRNA (cDNA) level are easier to perceive, since they do not differ in single nucleotides, as at the genomic DNA level, but in noticeable deletions (allele *F*: 111 bp, *D*: 33 bp, *G*: 39 bp) and a long insertion in *E* allele. Allele *0*, at cDNA level is associated with long deletion of approx. 8 kb at 3' end (Martin and Addeo, 1995).

The method described by Tokarska *et al.* (1999) gives a possibility of using blood and milk mononuclear cells as alternative sources of mRNA for αS1 casein variant studies and allows to avoid invasive mammary gland biopsy in order to obtain αS1 casein mRNA for analyses.

The presence of αS1 casein mRNA in milk is related, as we may guess, with the presence of mammary glands cells, but we also found, quite unexpectedly, that αS1 casein gene might express not only in mammary gland cells but also in blood via peripheral blood mononuclear cells. The presence of αS1 casein transcript in blood leukocytes brings about a possibility of detection of the gene polymorphism also in young females. Furthermore, since Szumska (1999) ascertained the presence of αS1 casein transcript in buck blood, it may serve as a source of αS1 casein mRNA in buck genotyping.

The main problem which occurred during two years of analyses was that all attempts of receiving RT-PCR products from samples collected during summer and autumn months (June–October) failed, while from samples collected during the first months of milking period (January–March) stable cDNA was regularly obtained. The problem concerned both milk and blood samples.

The results received by using both methods show that somatic cells of milk and peripheral blood leukocytes may be used as alternative sources of mRNA for analyses of genetic polymorphism of αS1 casein gene in goats and thus, what seems quite probable, also for other milk protein genes.

REFERENCES

Barbieri M.E., Manfredi E., Elsen J.M., Ricordeau G., Bouillon J., Grosclaude F., Mahé M.F., Bibé B. (1995): Influence du locus de la caséine αS1 sur les performances laitières et les paramètres génétiques des chèvres de race Alpine. Genet. Sel. Evol., *27*, 437–450.

Delacroix-Buchet A., Degas C., Lamberet G., Vassal L. (1996): Influence des variants AA et FF de la caséine αS1 caprine sur le rendement fromager et les caractéristiques sensorielles des fromages. Lait, *76*, 217–241.

Grosclaude F., Mahé M.F., Brignon G., Di Stasio L., Jeunet R. (1987): A Mendelian polymorphism underlying quantitative variations of goat αS1 casein. Génét. Sél. Evol., *19*, 399–412.

Leroux C., Martin P., Mahé M.F., Levéziel H., Mercier J.C. (1990): Restriction fragment length polymorphism identification of goat αS1 casein alleles: a potential tool in selection of individuals carrying alleles associated with a high level of protein synthesis. Anim. Genet., *21*, 342–351.

Leroux C., Mazure N., Martin P. (1992): Mutations away from splice site recognition sequences might *cis*–modulate alternative splicing of goat αS1–casein transcripts. J Biol. Chem., *9*, 6147–6157.

Manfredi E., Ricordeau G., Barbieri M.E., Amigues Y., Bibé B. (1995): Génotype caséine αS1 et sélection des boucs sur descendance dans les races Alpine et Saanen. Genet. Sel. Evol., *27*, 451–458.

Martin P., Addeo F. (1995): Genetic polymorphism of casein in the milk of goats and sheep. In: Int. Dairy Fed., 19–20 Oct. Crete, Greece. 45–58.

Pérez M.J., Leroux C., Sánchez A.S., Martin P. (1994): Occurrence of a LINE sequence in the 3' UTR of the goat αS1 casein E–encoding allele associated with reduced protein synthesis level. Gene, *147*, 179–187.

Remeuf F. (1993):. Influence du polymorphisme génétique de la caséine αS1 caprine sur les caractéristiques physico–chemiques et technologiques du lait. Lait, *73*, 549–557.

Ricordeau G., Piacere A., Manfredi E., Amigues Y. (1995): Fréquences alléliques de la caséine αS1 chez boucs d'insémination de race Alpine et Saanen de 1975 à 1994. INRA Prod. Anim., *8*, 259–264.

Szumska D. (1999):. Detection of molecular polymorphism of caprine alpha S1 casein as a main criterion in selection of bucks of milk breeds. [Ph. D. Dissertation.] Agricultural University in Wrocław, Poland.

Tokarska M., Szumska D., Dobosz T. (1999): RT-PCR – based identification of genetic variants of alpha S1 casein in milking goats. J. Appl. Genet., *4*, 335–342.

Vassal L., Delacroix–Buchet A., Bouillon J. (1994): Influence des variants AA, EE, FF de la caséine αS1 caprine sur le rendement fromager et les caractéristiques sensorielles de fromages traditionnels: premières observations. Lait, *7*, 89–103.

Walsh P.S., Netzger D.A., Higuchi R. (1991): Chellex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques, *10*, 506–513.

Received: 00–06–22
Accepted after corrections: 00–11–10

Corresponding Author:

Dr. Ing. Małgorzata Tokarska, adjunct, Department of Genetics and Animal Breeding, Agricultural University, ul. Kożuchowska 7, 51-631 Wrocław, Poland
Tel. +48 71 3205 759, fax +48 71 3205 758, e-mail: gosia@gen.ar.wroc.pl

Calcium, magnesium and phosphorus retention in young chicks

Retence vápníku, hořčíku a fosforu u mladých kuřat

J. ZELENKA, E. FAJMONOVÁ

Mendel University of Agriculture and Forestry, Faculty of Agronomy, Brno, Czech Republic

ABSTRACT: Utilisation of calcium, magnesium and phosphorus was examined in broilers and laying type cockerels from Day 3 to Day 22. Differences between the individuals birds with and without deutectomy were also studied. Utilisation of Ca and Mg increased very quickly and reached its maximum at 14 days of age. In intact chicks coefficients of apparent phosphorus retention decreased ($P < 0.01$) during the whole experiment while in deutectomized ones they increased till the age of 13 days; thereafter, a decrease was recorded. Retention of calcium per 1 g of weight gain in chicks with and without yolk sacs increased till Day 16–17 and during the whole experimental period ($P < 0.01$), respectively. Content of magnesium in weight gains increased and culminated on Day 13–14. Concentration of phosphorus in weight gains in chicks with and without yolk sacs increased till Day 14 and till the end of experiment, respectively. Differences in Ca, Mg and P retention per unit of weight gain in laying type hybrids and in broiler chicks were not significant ($P > 0.05$).

Keywords: chick; calcium; magnesium; phosphorus; retention of macrominerals

ABSTRAKT: U kohoutků masného a nosného typu jsme od 3. do 22. dne života sledovali využití vápníku, hořčíku a fosforu. Zjišťovali jsme také rozdíly mezi deutektomovanými kuřaty a kohoutky s ponechanými žloutkovými vaky. Využití Ca a Mg se velmi rychle zvyšovalo a dosáhlo maxima ve 14 dnech věku. Koeficienty bilanční retence fosforu se u intaktních kuřat během celého experimentu snižovaly ($P < 0,01$), zatímco u deutektomovaných kuřat do věku 13 dní vzrůstaly a teprve potom začaly klesat. Retence vápníku v přírůstku živé hmotnosti se u kuřat se žloutkovými vaky zvyšovala do 16.–17. dne a u deutektomovaných kuřat během celého sledovaného období ($P < 0,01$). Počáteční růst koncentrace hořčíku v přírůstcích kulminoval ve 13.–14. dni života. Koncentrace P v přírůstcích se u intaktních kuřat zvyšovala pouze do 14. dne, zatímco u deutektomovaných kuřat až do konce našeho sledování. Rozdíly v retenci Ca, Mg a P na jednotku přírůstku živé hmotnosti u kuřat masného a nosného typu nebyly průkazné ($P > 0,05$).

Klíčová slova: kuřata; vápník; hořčík; fosfor; retence makroprvků

INTRODUCTION

For the formulation of starter feed mixtures for chicks it is necessary to know what changes in nutrient utilisation take place during the period of early growth. We have not found any references to daily changes in macromineral utilisation within the first days of postembryonal life.

In case that the level of calcium in feed is sufficient, intensity of its absorption is determined above all by the animal's requirement. In an experiment conducted by Zelenka (1991a), the effect of chicken age on calcium utilisation was studied in 240 balance experiments with chicks receiving a practical type of feed mixture containing 6.8 or 8.4 g of Ca per 1 kg of dry matter from the 12th to the 56th day of age. The linear decrease in utilisation of Ca originating from a feed mixture with the higher level of this element was highly significant during the whole experimental period. On the other hand, utilisation from a mixture with the suboptimum level of Ca increased gradually with the increasing age until the amount of retained Ca was equal to that from the mixture rich in Ca; thereafter, it began to decrease untill the end of experiment. This suggests that the organism adapted itself gradually to the utilisation of Ca in dependence on a different supply of this element to satisfy the total Ca requirement.

This study was supported by the Ministry of Agriculture of the Czech Republic (Grant No. 6186/96).

In our previous experiment (Zelenka, 1992), the percentage of utilisation of magnesium from feed mixtures decreased with the increasing age of chickens but the content of Mg in weight gains increased highly significantly during the whole fattening period from the 12th day of age to the end of fattening. The increase was linear.

The intensity of P retention depends on the requirements of the chicks. Phosphorus requirement decreases with increasing age of chicks (Simons, 1979). In our another experiment (Zelenka, 1987), the retention of P per unit of weight gain highly significantly decreased with age when the feed mixture was rich in this element (6.5 g per kg dry matter) while when feed contained only 5.1 g of P per 1 kg of dry matter the concentration in weight gains did not change.

MATERIAL AND METHODS

Effect of age on the apparent calcium, magnesium and phosphorus retention was investigated within subsequent one-day balance periods using 182 Isa Brown laying type cockerels and 52 Ross 208 meat type intact (YS+) male chicks. In 87 Isa Brown birds, yolk sacs were removed immediately after hatching (YS–) while the remaining 95 cockerels were intact (YS+). In Isa Brown, observations were carried out from the time of hatching to the end of experiment on Day 22. In Ross 208, observations covered the period from Day 1 of age to Day 22. For details about the environment including the composition of the diet see our paper about fat digestibility and nitrogen retention (Zelenka *et al.*, 2000). The diet contained 8.63 g Ca, 0.60 g Mg and 6.16 g P per kg. The feed was supplied *ad libitum*. The coefficients of utilisation of macrominerals under study were estimated using the chromic oxide indicator method. The content of chromic oxide in feed and freeze-dried excreta was estimated iodometrically (Mandel *et al.*, 1960). Calcium, magnesium and phosphorus were estimated after dry mineralization (APION, Tessek Prague, Ltd., 25°C – 400°C, oxidative medium $O_3 + NO_x$), Ca and Mg by the flame atomic absorption spectrometry using Varian SpectrAA-30 Atomic Absorption Spectrometer at wavelengths 422.7 nm (Ca) and 202.6 nm (Mg) and P spectrophotometrically as vanadate yellow at wavelengths 430 nm (Regulation No. 222/1996 of the Czech Ministry of Agriculture). There were three replications in each determination. The differences between parallel estimations were lower than 3 relative per cent. Our results on reference materials (feed mixtures for poultry for interlaboratory tests of the Central Institute for Supervising and Testing in Agriculture) were in agreement with certified values. The recoveries for Ca, Mg and P were 91.21 ± 1.477, 86.33 ± 0.425 and 97.98 ± 0.866 per cent, respectively (mean ± standard error of the mean; 20 determinations).

The amount of consumed feed from which elements deposited in the body originated was calculated on the basis of chromic oxide concentration in feed mixture and excreta and the amount of quantitatively collected excreta. The contents of Ca, Mg and P in the live weight gain (mg/g) were calculated using feed intake determined by the method described above, contents of macrominerals in the diet, coefficients of its utilisation and last day weight gain.

The regressions of determined values were computed according to Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Surgically removed yolk sacs contained 32.5 mg Ca, 2.1 mg Mg and 9.4 mg P on the average.

In all Isa Brown chicks, more Ca, Mg and P was found in the excreta than was the intake of these elements on the first day after hatching. The same was observed in deutectomized chicks (YS–) with P on Day 2 of age. Isa Brown YS+ birds excreted more Ca and Mg and less P than Isa Brown YS– (Tab. 1).

The mean values of macromineral apparent retention coefficients and retention per one gram of weight gain are presented in Tab. 2. Dependences of the above values (Y) on the age of chicks in days (X) within the period of Day 3 to Day 22 were expressed by means of linear regression equations and the 2nd degree parabola equations. The reduction in the sum of squares of deviations was tested against the mean square remaining after curvilinear regression by *F*-test (Snedecor and Cochran, 1967). In case that the reduction was significant, parameters *a*, *b* and *c* of the parabol equation were presented in Tab. 2. When the deviation from linearity was insignificant, parameters *a* and *b* of linear regression were presented.

Dependence of coefficients of apparent calcium retention on the age of chicks was significant and expressed by means of the 2nd degree parabolas with maxima on Day 14–15 (Fig. 1). The content of Ca in the diet was excessively high from this age and that was the reason why the utilisation decreased similarly like in our previous experiment (Zelenka, 1991a) when rich-in-Ca diet was fed.

Vogt *et al.* (1985) reported that the newly hatched chick contained 4.3 mg Ca and a broiler at the age of 3 weeks 6.8 mg Ca per 1 g of live weight. In our experiment the retention of Ca per 1 g of weight gain in chicks with yolk sacs increased till Day 16–17 and in deutectomized chicks during the whole experimental period from Day 3 to Day 22 (Tab. 2). The average contents of Ca in 1 g of weight gain of broilers, laying chicks YS+ and laying chicks YS– were 5.48 ± 0.314, 6.48 ± 0.474 and 6.73 ± 0.578 mg, respectively. The differences between the groups of chicks were not significant ($P > 0.05$).

Utilisation of magnesium increased very quickly since the beginning of postembryonal life (Fig. 2). At the age

Table 1. Intake and excretion of calcium, magnesium and phosphorus

Element	Age of chicks in days	Intake in mg			Excretion in mg		
		Isa Brown		Ross 208	Isa Brown		Ross 208
		YS+	YS–	YS+	YS+	YS–	YS+
Calcium	1	0.5	0.2	–	3.4	0.3	–
	2	5.9	3.5	23.1	4.1	1.6	13.6
	3	19.6	15.0	69.7	12.3	11.2	45.5
	4	32.0	22.8	95.0	19.3	12.2	56.4
Magnesium	1	0.1	0.05	–	0.6	0.3	–
	2	1.5	0.9	5.9	1.4	0.7	4.6
	3	5.0	3.8	17.7	4.4	2.6	14.1
	4	8.1	5.8	24.2	6.2	4.0	16.3
Phosphorus	1	0.4	0.1	–	0.4	3.3	–
	2	4.6	2.8	18.0	1.9	3.5	8.0
	3	15.3	11.7	54.5	7.9	8.9	29.0
	4	25.0	17.8	74.3	14.1	13.5	45.0

YS+ = chicks with yolk sacs
YS– = deutectomized chicks

of 14 days, it was the highest and more than 50 and 46 per cent of consumed Mg was retained in the body of intact and deutectomized chicks, respectively. Thereafter, a marked decrease could be observed that was followed by a period of stabilized values ranging between 12 and 33 per cent. This marked decrease in Mg utilisation, as observed at the beginning of the third week of age, is difficult to explain; however, the same phenomenon was also observed in our earlier experiment with Mg utilisation in 16 groups of broilers in three-day balance periods at the age of 12 to 56 days of life. The measured values were 38.34 ± 1.559 and 26.39 ± 1.040 per cent in the first

Table 2. Apparent retention of calcium, magnesium and phosphorus

Mean and regression equation			Mean ± standard error of the mean	$Y = a + bX (+ cX^2)$						
				a	b	c	r	P	$X_{extr.}$	$Y_{extr.}$
Coefficients of retention	Calcium	Isa Brown YS+	47.81 ± 1.867	27.4	3.52	–0.1242	0.538	< 0.05	14.2	52.28
		Isa Brown YS–	45.50 ± 1.681	19.9	4.26	–0.1462	0.760	< 0.01	14.6	50.99
		Ross 208 YS+	47.85 ± 1.222	27.5	3.61	–0.1310	0.816	< 0.01	13.8	52.42
	Magnesium	Isa Brown YS+	32.32 ± 2.875	0.5	7.38	–0.3185	0.800	< 0.01	11.6	43.18
		Isa Brown YS–	31.38 ± 2.138	25.1	2.81	–0.1520	0.782	< 0.01	9.2	38.06
		Ross 208 YS+	37.07 ± 2.155	15.9	5.17	–0.2293	0.801	< 0.01	11.3	45.04
	Phosphorus	Isa Brown YS+	35.99 ± 1.543	48.0	–0.96**	–	0.821	> 0.05	–	–
		Isa Brown YS–	30.15 ± 1.454	10.7	3.70	–0.1414	0.678	< 0.01	13.1	34.90
		Ross 208 YS+	37.94 ± 1.504	47.8	–0.79**	–	0.696	> 0.05	–	–
Retention per 1 g of weight gain	Calcium (mg)	Isa Brown YS+	6.48 ± 0.474	–1.11	1.180	–0.037785	0.851	< 0.01	15.6	8.10
		Isa Brown YS–	6.73 ± 0.578	2.18	0.364**	–	0.834	> 0.05	–	–
		Ross 208 YS+	5.48 ± 0.314	0.25	0.764	–0.022785	0.954	< 0.01	16.8	6.65
	Magnesium (mg)	Isa Brown YS+	1.12 ± 0.122	–0.68	0.360	–0.014271	0.794	< 0.01	12.6	1.60
		Isa Brown YS–	1.10 ± 0.067	0.40	0.134	–0.005147	0.535	< 0.05	13.0	1.27
		Ross 208 YS+	1.06 ± 0.070	–0.09	0.216	–0.008184	0.818	< 0.01	13.2	1.34
	Phosphorus (mg)	Isa Brown YS+	3.64 ± 0.211	0.41	0.601	–0.022592	0.762	< 0.01	13.3	4.40
		Isa Brown YS–	3.43 ± 0.273	1.56	0.150**	–	0.725	> 0.05	–	–
		Ross 208 YS+	3.30 ± 0.156	0.90	0.434	–0.015966	0.756	< 0.01	13.6	3.85

X = age in days
a, b, c = parameters of equation
r = correlation coefficients
P = significance of the deviation from linearity
** significance of linear regression ($P < 0.01$)

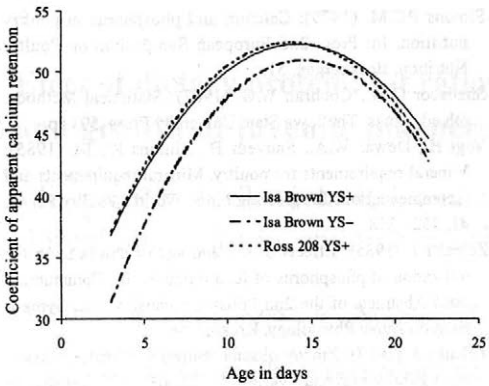

Figure 1. Calcium retention

(Days 12 to 14) and the second (Days 15 to 17) balance periods, respectively; the difference was highly significant ($P < 0.001$; Zelenka, 1992).

In the present experiment, the difference in Mg utilisation in chicks of different genotypes was not significant ($P > 0.05$).

Vogt *et al.* (1985) mentioned that immediately after hatching chicks contained 0.24 mg Mg per 1 g of live weight. In our experiment Mg content in live weight gains increased gradually from initial ca 0.27–0.75 mg/g to maximum values of 1.27–1.60 mg/g which were observed on Day 13 or 14. During the following two or three days these values decreased to a lower level and ranged about 0.9 mg/g of weight gain till the end of experiment.

Chickens with a lower growth rate accumulated significantly ($P < 0.001$) more Mg per unit of weight gain than those with a higher growth rate in an experiment conducted by Zelenka (1991b). In the present experiment, the contents of Mg in weight gains were practically the same in chicks of laying hybrid combination and in faster-growing broiler chicks.

In deutectomized chicks, utilisation of P was highly significantly ($P < 0.01$) lower (30.15 ± 1.454 per cent) than in intact birds of hybrid combination Isa Brown (35.99 ± 1.543 per cent) and Ross 208 (37.94 ± 1.504 per cent). Deutectomy probably disturbed the mechanism of phosphorus absorption from feed and its excretion. Through active absorption the percentage of absorbed P increases in response to an increased demand for P (McDowell, 1992). In our experiment retention of P increased till the age of 13 days and then it began to decrease in chicks without yolk sacs (Fig. 3). In intact chicks, utilisation of phosphorus decreased in the whole experimental period from Days 3 to 22 of age, in Isa Brown by 0.96 per cent and Ross 208 by 0.79 per cent daily. The decrease was highly significant ($P < 0.01$). In our another experiment (Zelenka, 1985), retention also highly significantly decreased in the period of Day 12 to Day 56. The dependence of phosphorus utilisation on age was expressed by the sloping part of convex parabola.

The level of dietary phosphorus in the present experiment was not probably sufficient for chicks without yolk sacs till Day 14 and for that reason the utilisation of this element was increased through active transport. Phosphorus requirement decreases with the increasing age of animals and this is the reason why the utilisation of dietary P in older chicks decreased. Cockerels without deutectomy have phosphorus at their disposal not only from the feed but also extra phosphorus from the yolk sac; therefore the utilisation of P in connection with declining requirement decreased for the whole period under study.

The dependence of P contents (mg) per 1 g of weight gain on age (in days) was expressed by the second degree parabolas for intact chicks

Ross 208 $Y = 0.90 + 0.434\,X - 0.015966\,X^2$; $r = 0.756$; $P < 0.01$ and

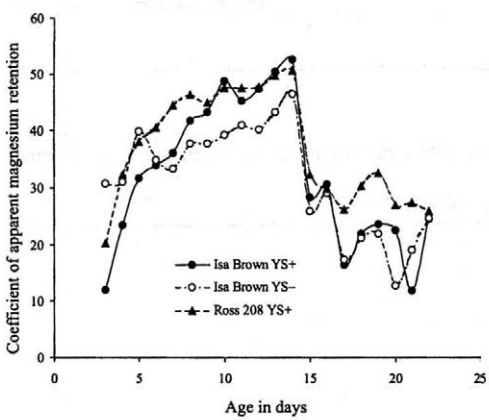

Figure 2. Magnesium retention

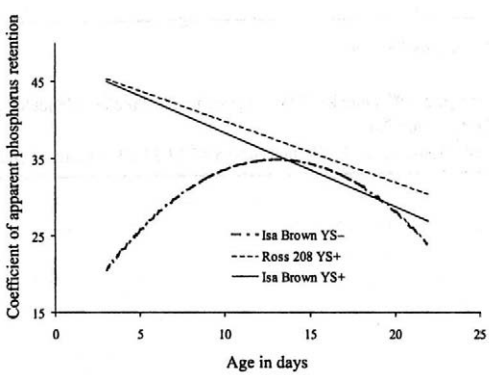

Figure 3. Phosphorus retention

Isa Brown $Y = 0.41 + 0.601\,X - 0.022592\,X^2$; $r = 0.762$; $P < 0.01$

with maximum values on Day 14 of age. A parabolic dependence of phosphorus retention on age confirmed the results of an experiment carried out by Jermenkov and Kharukchijeva (1978), who observed the incorporation of labelled phosphorus into RNA of chicken liver cells. Incorporation increased from Day 10 till Day 17 and thereafter went down. The content of P in deutectomized Isa Brown cockerels linearly increased by 0.15 per cent daily; this increase was highly significant ($P < 0.01$). The difference in average contents of P in live weight gains in laying type hybrids (3.64 ± 0.211 mg/g) and in broiler chicks (3.30 ± 0.156 mg/g) was not significant ($P > 0.05$). In our previous experiment (Zelenka, 1988), chickens with a lower growth rate accumulated significantly ($P < 0.01$) more P per unit weight gain than those with a higher growth rate. Different strains or breeds of poultry have different requirements for certain nutrients. The Leghorn chick was found by McDowell (1992) to have a higher requirement for P than does the broiler chick.

REFERENCES

Jermenkov K.I., Kharukchijeva S.B. (1978): Influence of thyroxine and iodine ions on the dynamics of incorporation of ^{32}P in the RNA of the liver in chicks. Doklady Bolg. Akad. Nauk, *31*, 1347–1350.

Mandel L., Turynek V.,Trávníček J. (1960): Jodometrická metoda stanovení kysličníku chromitého, použitého jako indikátoru při pokusech stravitelnosti (An iodometric method of determination of chromic oxide, used as an indicator in digestibility trials). Živoč. Výr., *5*, 645–652.

McDowell L.R. (1992): Minerals in Animal and Human Nutrition. San Diego, Academic Press. 524 pp.

Simons P.C.M. (1979): Calcium and phosphorus in poultry nutrition. In: Proc. 2nd European Symposium on Poultry Nutrition, Beekbergen, 51.

Snedecor G.W., Cochran W.G. (1967): Statistical Methods. 6th ed. Ames, The Iowa State University Press. 593 pp.

Vogt H., Dewar W.A., Sauveur B., Simons P.C.M. (1985): Mineral requirements for poultry. Mineral requirements and recommendations for growing birds. World's Poultry Sci. J., *41*, 252– 258.

Zelenka J. (1985): Effects of sex and age of chicks upon the utilization of phosphorus of feed mixtures. In: Communications Abstracts of the 2nd Polish-Czechoslovakian Symposium on Avian Physiology, Kraków, 54.

Zelenka J. (1987): Změny obsahu fosforu v přírůstcích hmotnosti kuřat v průběhu výkrmu. In: Zborník z XI. celoštátnej konferencie. Racionalizácia výroby a využitia krmív, kŕmnych zmesí a doplnkov vo výžive hospodárskych zvierat. Štrbské Pleso, ČSVTS, 296–299.

Zelenka J. (1988): Retention of phosphorus in the organism of fattened chicks. In: Proc. 21st International Conference on Poultry Physiology. Prague, University of Agriculture, 184–185.

Zelenka J. (1991a): Effect of calcium level in feed mixtures on its utilization in chicks during the fattening period. In: Proc. 8th European Symposium on Poultry Nutrition. Venezia-Mestre, World's Poultry Science Association, 311–313.

Zelenka J. (1991b): Retention of magnesium in the organism of fattened chicks. In: Proc. 24th International Conference on Poultry Physiology. Brno, University of Agriculture, 6–7.

Zelenka J. (1992): Utilization of magnesium in chicks during the fattening period. In: Proc. 19th World's Poultry Congress, Vol. 1. Amsterdam, World's Poultry Science Association, 626.

Zelenka J., Fajmonová E., Blažková E. (2000): Apparent digestibility of fat and nitrogen retention in young chicks. Czech J. Anim. Sci., *45*, 457–462.

Received: 00–08–14
Accepted after corrections: 00–11–10

Corresponding Author:

Prof. Ing. Jiří Zelenka, CSc., Agronomická fakulta, Mendelova zemědělská a lesnická univerzita, Zemědělská 1, 613 00 Brno, Česká republika
Tel. +420 5 45 13 31 59, fax +420 5 45 13 31 99, e-mail: zelenka@mendelu.cz

Efect of dietary protein: fat ratios on metabolism, body composition and growth of juvenile pikeperch, *Stizostedion lucioperca* (L.)

Vliv poměru dusíkatých látek a tuku v krmné směsi na metabolismus, složení těla a růst juvenilního candáta, *Stizostedion lucioperca* (L.)

Z. Zakes[1], M. Szkudlarek[1], M. Wozniak[2], A. Karpinski[1], K. Demska-Zakes[2]

[1]*The Stanislaw Sakowicz Inland Fisheries Institute, Olsztyn-Kortowo, Poland*
[2]*University of Varmia and Mazuria, Olsztyn-Kortowo, Poland*

ABSTRACT: The effects of diet composition (commercial trout feeds: 42.2–52.5% protein, and 14.0–22.0% fat) on growth rate, oxygen consumption, ammonia excretion and body composition were examined in juvenile pikeperch (initial total length 16.5 cm, and body weight 42.5 g) reared in a water recirculation system for 28 days from day 128 to 156 post-hatch. There were no significant differences in fish growth, condition, ammonia excretion (AE) and ammonia quotient (AQ) between the experimental groups ($P > 0.05$). Mean oxygen consumption (OC) was within the range 195.50–211.14 mg O_2/kg/h, and the differences between the groups were statistically significant ($P < 0.05$). Dietary protein concentration had no significant effect on the protein concentration of the fish, but dietary fat concentration had a significant influence on the fat concentrations in the fish ($P < 0.05$), which ranged from 6.6 to 8.5% of pikeperch body wet-weight.

Keywords: *Stizostedion lucioperca* L. (Percidae); metabolism; body composition; growth; nutritional status

ABSTRAKT: Vliv složení krmiva (komerční krmiva pro pstruhy: 42,2 až 52,5 % dusíkatých látek a 14,0 až 22,0 % tuku) na intenzitu růstu, spotřebu kyslíku, vylučování amoniaku a složení těla jsme sledovali u juvenilního candáta *Stizostedion lucioperca* (L.), o počáteční celkové délce 16,5 cm a tělesné hmotnosti 42,5 g. Odchov probíhal za recirkulace vody po dobu 28 dní mezi 128. a 156. dnem po vylíhnutí. Mezi pokusnými skupinami ($P > 0,05$) jsme nezjistili žádné významné rozdíly v růstu a vyživenosti ryb, vylučování amoniaku (AE) a koeficientu amoniaku (AQ). Průměrná spotřeba kyslíku (OC) se pohybovala mezi 195,50 až 211,14 mg O_2/kg/h. Rozdíly mezi jednotlivými skupinami byly statisticky významné ($P < 0,05$). Koncentrace dusíkatých látek v krmivu neovlivnila významně koncentraci dusíkatých látek v těle ryb, ale koncentrace tuku v krmivu měla významný vliv na koncentraci tuku v rybách ($P < 0,05$), která se pohybovala od 6,6 do 8,5 % tělesné hmotnosti candáta v čerstvé hmotě.

Klíčová slova: *Stizostedion lucioperca* L. (Percidae); metabolismus; složení těla; nutriční stav

INTRODUCTION

Many carnivorous fish, such as salmonids (*Oncorhynchus* spp.) grow well when about half of the energy contained in the food is supplied as protein, whereas the protein requirements of commercially important omnivorous fish species, such as carp, *Cyprinus carpio* (L.) and tilapia (*Oreochromis* spp.), are lower, and rapid growth of these fish may be ensured when diets contain 30–40% protein (Jobling, 1994).

Pikeperch, *Stizostedion lucioperca* (L.), is a carnivorous species that may become the object of intensive aquaculture in the future, but it is not expected that feeds will be formulated specially for the species in the first instance. Thus, an early step in initiating culture of this fish is to examine the possibility of using feeds produced for other species, such as salmonids. Trout pellets may be used to raise 0+ pikeperch (Zakes and Demska-Zakes, 1998), and pikeperch reared on commercial trout feeds for 720 days, in a water recirculation system, attained weight in excess of 1 kg (Zakes *et al.*, unpubl. data).

The objective of this study was to examine the feasibility of using commercial trout feeds to rear juvenile

pikeperch, using assessments of growth rate, metabolism and body composition as performance criteria.

MATERIAL AND METHODS

Source of fish and rearing conditions

Pikeperch fry obtained by the artificial spawning of broodstock (♀–2.5 kg; 2♂–1.0–1.5 kg) held at the "Dgal" Hatchery of the Inland Fisheries Institute, Olsztyn (located in Mazuria, northern Poland) were reared on a mixed food (formulated diet + *Artemia* sp.) for three weeks, and on trout pellets thereafter.

On day 123 post hatch (D123) groups of 51 fish were stocked into 0.2 m^3 tanks within two independent water recirculation systems. Water flow was established at 4 L/min (exchange rate 1.2/h), and temperature was 21.7 ± 0.1°C. The tanks were exposed to continuous light, and intensity just above the water surface was 40–50 lx. Replicated tanks of fish were provided with one of the three commercial extruded trout feeds (pellet size 2 mm). The feeds differed as regards chemical composition, although gross energy was calculated to be similar 20.1–22.6 MJ/kg (Tab. 1). Feeds were distributed at a rate of 1.5% tank biomass using automatic band feeders for 18 hours daily (09.00–03.00 hours). The daily feed ration was expected to be in excess of consumption, and this was confirmed by the observation of feed waste. Feeds were introduced for an adaptation period of five days, during which it was observed that the feeds were readily consumed. The experiment, which lasted for four weeks, was initiated on day 128. Mean body weight of the fish at the start of the experiment was 42.5 g, and total length was 16.5 cm (Tab. 2), and stocking densities were 10.6 kg/m^3 (diet C) to 10.8 kg/m^3 (diet A). Fish tanks were cleaned every day, an hour before feeding commenced (08.00 hours). Water quality was monitored every day. Total ammonia nitrogen (TAN = NH_4^+-N + NH_3-N) did not exceed 0.15 mg TAN/L, oxygen concentration did not drop below 5.4 mg/L, and nitrite (NO_2-N) in the outflow did not exceed 0.018 mg/L. Water pH was 7.52 and 7.47 in the inflow and outflow, respectively.

Table 1. Proximate analysis (percent weight composition) and energy of the diets (A, B, C) used for pikeperch rearing

	Diet		
	A	B	C
Composition			
Crude protein	52.5	42.2	51.1
Crude fat	14.0	21.8	22.0
Moisture	9.9	9.3	6.5
Ash	10.1	6.5	9.0
Fibre	1.7	2.0	1.5
Nitrogen-free extract	11.8	18.2	9.8
Energy (MJ/kg diet)			
Gross (GE)*	20.1	21.7	22.6
Protein E : Gross E	0.62	0.46	0.53

* based on the values 24 kJ/g protein, 39 kJ/g fat and 17 kJ/g carbohydrate (Jobling, 1994)

In order to determine growth rate, food conversion ratio [FCR = weight of the feeds used (kg)/fish biomass increment (kg)] and food doses, a random sample of 20 fish was collected at the start and every 7 days of the experiment from each tank. The fish were measured (TL ± 0.1 mm) and weighed (BW ± 0.01 g). Condition factors (K) were calculated as: K = [(body weight (g) × 100)/TL^3 (cm)]. Total fish biomass was determined weighing all fish in a container with the known water weight.

Measurements of metabolic rates

Oxygen consumption (OC) and ammonia excretion (AE) were monitored like in earlier studies (Zakes and Karpinski, 1999) on days 145 and 147 (OC) and day 147

Table 2. Growth and condition data [means] of pikeperch fingerling reared on three different diets. Means with the same superscripts in the same column are not significantly different ($P > 0.05$)

Diet (number of observations)	Sample time (day after hatching)						Growth per day (D128–D156)	
	D128 (beginning of treatment)			D156 (end of treatment))				
	Total length TL (cm)	Body weight (g)	Condition factor K	Total length TL (cm)	Body weight (g)	Condition factor K	(mm/d)	(g/d)
A $N = 2$	16.82^A	42.44^A	0.89^A	19.35^A	62.89^A	0.87^A	0.9	0.73
B $N = 2$	16.74^A	42.41^A	0.90^A	19.23^A	63.10^A	0.89^A	0.9	0.74
C $N = 2$	16.46^A	41.63^A	0.93^A	19.38^A	64.76^A	0.89^A	1.0	0.83

(AE), respectively. Checks were also carried out using tanks without fish. In these blank trials the differences in oxygen saturation between inflow and outflow water (water flow was the same as in the rearing tanks) were lower than 1%, so rates of oxygen consumption by microorganisms and/or oxygen diffusion were considered negligible. Ammonia quotient (AQ) was calculated as: Ammonia excretion/Oxygen consumption (mole to mole relation); ammonia excretion expressed as mg TAN/kg/h, oxygen consumption as mg O_2/kg/h.

Fish size and biomass were measured at the end of the metabolic rate measurements (day 147). Tank loading was 0.7 kg of fish/L/min and stock density amounted to 14 kg/m^3. Mean total length (cm) and body weights (g) were respectively: 18.36 and 54.92 (diet A), 18.35 and 54.03 (diet B), and 18.33 and 55.76 (diet C).

Body composition

At the start of the experiment (D128) ten fish were sampled for proximate composition analysis, and at the end of the study (D156) ten fish from each tank were sampled. The fish (from each tank) were pooled prior to analysis, then were minced, homogenised, and lyophilised. Dry weight, crude protein (Kjeldahl method), fat (Soxhlet method) and ash were determined using standard procedures (Skulmowski, 1974). Chemical composition of the diets was also analysed. In order to determine the differences in body composition of the fish fed artificial diets and those consuming natural food, additional determinations were performed of body composition in the fish of the same age but different size (13.9 cm TL, 16.0 g BW) collected on October 30 from fish ponds on Grzmieca Fish Farm (central Poland).

To establish the significance of differences ($P < 0.05$) in growth, metabolic rates and chemical composition of the fish on different diets, one-way variance analysis (ANOVA) was used together with Duncan's test. Mean percentage of body composition was arcsine-transformed before analysis.

RESULTS

Fish metabolism

Mean oxygen consumption was within the range 196.50 mg O_2/kg/h (diet A) to 211.14 mg O_2 /kg/h (diet B), and the differences were statistically significant ($P < 0.05$; Tab. 3). Different diets had no effect on the mean levels of ammonia excretion, which ranged from 6.41 mg TAN/kg/h (diet B) to 6.98 mg TAN/kg/h (diet A). Also ammonia quotients (AQ) did not differ between the experimental groups ($P > 0.05$; Tab. 3). However, when AQ calculated for diet A and diet C only (extreme values) were analysed, statistically significant differences were found ($P = 0.029$). AE was directly proportional to OC, and the regression equations were highly significant, but specific for diet groups:

AE = 0.101 × OC – 12.92 ($r = 0.9517$; number of observations $N = 48$; diet A);

Table 3. Mean rates of oxygen consumption (mg O_2/kg/h) and ammonia excretion (mg TAN/kg/h) of pikeperch fingerlings fed three different diets

Diet (number of observations)	Metabolic rates		
	Mean*	Maximum – minimum**	Increase in maximum from mean (%)
	Oxygen consumption		
A ($N = 4$)	196.50^{A}	230.43–157.28	17.3
B ($N = 4$)	211.14^{B}	264.58–160.88	25.3
C ($N = 4$)	205.42^{AB}	230.52–158.20	12.2
P value	*0.0469*		
	Ammonia excretion		
A ($N = 2$)	6.98^{A}	9.58–2.39	37.3
B ($N = 2$)	6.41^{A}	10.09–1.76	57.4
C ($N = 2$)	6.91^{A}	9.75–2.47	41.1
P value	*0.5023*		
	Ammonia quotient		
A ($N = 2$)	0.079^{A}	0.102–0.033	28.5
B ($N = 2$)	0.068^{A}	0.100–0.025	48.1
C ($N = 2$)	0.075^{A}	0.102–0.034	35.0
P value	*0.0710*		

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

** maximum and minimum values are means of four (OC) and two (AE and AQ) experimental trials (replicates)

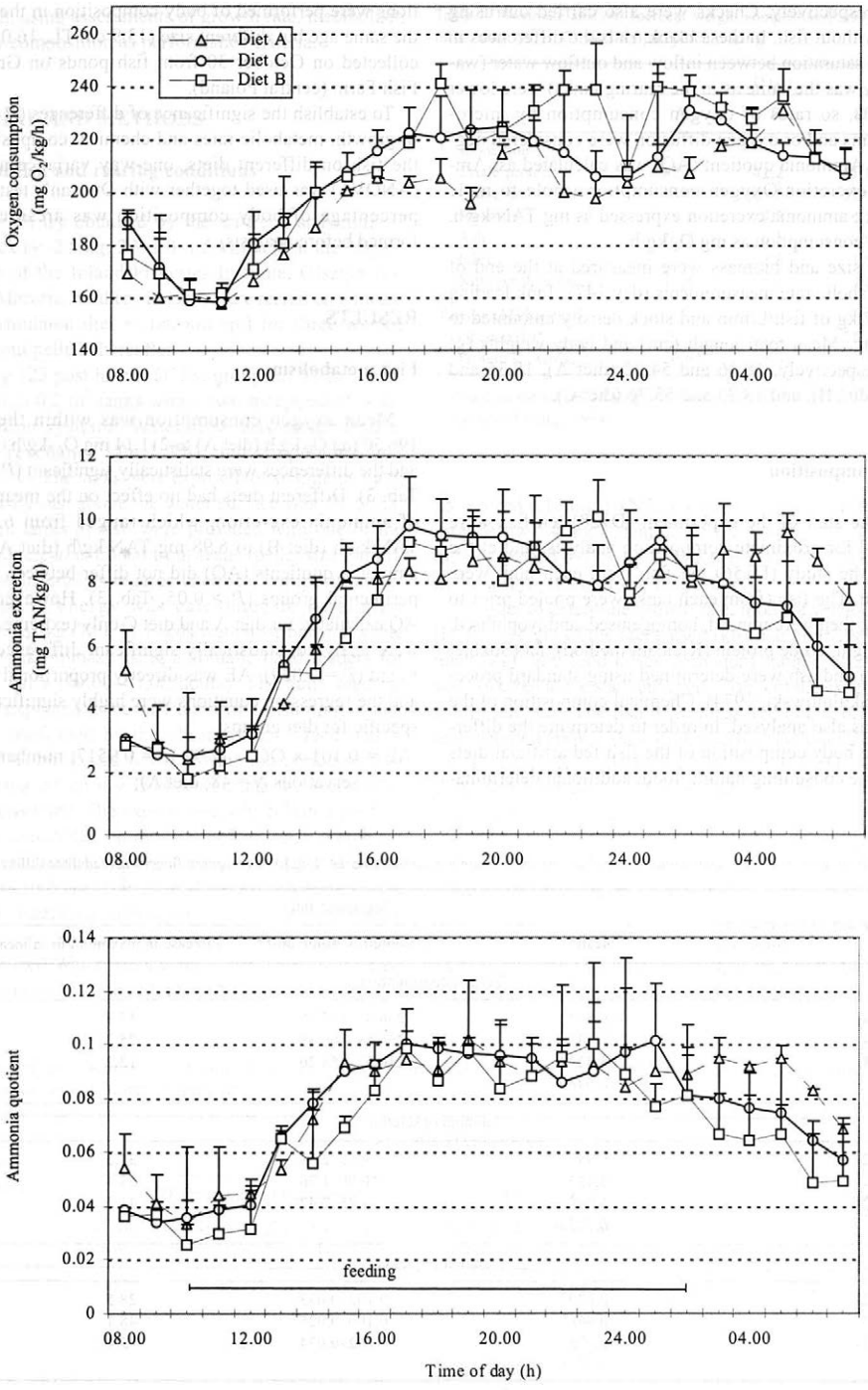

Figure 1. Diurnal variations in oxygen consumption, ammonia excretion and ammonia quotient (mean ± SD) in juvenile pikeperch

AE = 0.087 × OC – 11.85 (r = 0.8853; N = 48; diet B);
AE = 0.108 × OC – 15.21 (r = 0.8881; N = 48; diet C).

Daily profiles of OC and AE were very similar in all groups (Fig. 1). Intensive increase of the two parameters was observed 3 hours after the beginning of feeding. They increased rapidly for about 5 hours (8 hours after the beginning of feeding), and then stabilised. As soon as feeding was stopped, OC and AE values decreased rapidly, attaining the levels before feeding. The ratio between maximal oxygen consumption or ammonia excretion and mean daily values of these parameters was 1.12–1.25 (OC) and 1.37–1.57 (AE) (Tab. 3).

Body composition

There were no statistically significant differences in protein, ash and moisture levels between the groups of fish fed any of the three diets ($P > 0.05$). Mean values of these parameters were 17.7, 3.5 and 70.6% (Tab. 4).

Diet composition was reflected in fat concentrations of the fish body. They ranged from 6.6% (diet A) to 8.5% (diet B), and the differences between the groups were statistically significant ($P < 0.05$; Tab. 4). Comparing chemical composition of the reared fish fed commercial diets with that of fish collected from natural environment attention should be paid to fat concentration, about 7 times higher in fish on artificial food. Fish collected from natural conditions had lower protein and ash levels and higher moisture, but the differences were far less pronounced than in the case of fat (Tab. 4).

Table 4. Body composition of juvenile pikeperch fed three commercial diets.The values are given on wet-weight basis (N = 2 per treatment). Data with the same superscripts in the same column are not significantly different ($P > 0.05$). The respective initial values (D128) for crude protein, fat, ash and moisture were as follows: 17.0, 7.5, 3.2 and 71.9

Diet	Proximate analysis			
	Crude protein	Crude fat	Ash	Moisture
A	18.1^{A}	6.6^{A}	3.5^{A}	71.2^{A}
B	17.4^{A}	8.5^{B}	3.6^{A}	70.1^{A}
C	17.5^{A}	8.1^{B}	3.5^{A}	70.5^{A}
Pooled data	17.7	7.7	3.5	70.6
Natural feed	15.9	1.1	2.6	78.8

Growth

Average daily length and body weight increment in the period D128 – D156 was about 1.0 mm and 0.7–0.8 g. At the end of the experiment the mean body length and weight were from 19.23 cm (diet B) to 19.38 cm (diet C) and from 62.89 g (diet A) to 64.76 g (diet C) respectively (Tab. 2). Differences between the groups were not significant ($P > 0.05$).

Food conversion ratios of the diets were fairly similar, amounting to 0.94 (diet A), 0.93 (diet B), and 0.84 (diet C). Fish biomass at the end of the experiment amounted to: 16.04 kg/m^3 (diet A), 16.09 kg/m^3 (diet B) and 16.61 kg/m^3 (diet C). Hence, total increments of the fish biomass during the period D128 – D156 were 5.2 kg/m^3, 5.3 kg/m^3 and 5.9 kg/m^3, respectively.

DISCUSSION

The highest rate of oxygen consumption was observed in the fish group given diet with the lowest protein to energy ratio. This may suggest that fish in this group used protein in the most efficient way, while energy needed for various life functions originated from fat to a higher extent. On the other hand, it is known that despite large differences between energy content in proteins and fats, 1 L of consumed oxygen results in similar levels of energy released from these nutritive substances (Schmidt-Nielsen, 1994). In view of the fact that all growth parameters, with the exception of body composition, were similar in all fish groups, the observed differences in OC might have been related to different metabolic effect of the diets (SDA effect). A relationship between diet composition and SDA effect was already confirmed for trout, *Oncorhynchus mykiss* (Walbaum) (LeGrow and Beamish, 1986) and plaice, *Pleuronectes platessa* L. (Jobling and Davies, 1980). No measurements were made of OC in starved fish, so it was not possible to determine the SDA effect to confirm this suggestion.

The higher proportion of fats and/or carbohydrates as energy sources should have been reflected in ammonia excretion, as ammonia is the basic end product of protein metabolism, i.e. amino acids deamination (Schmidt-Nielsen, 1994). Ammonia excretion in fish with the highest OC was in fact at the lowest level, and differences between the groups proved to be statistically significant. Many studies suggest that AE increases with increasing amounts of consumed proteins (Kaushik, 1980; Forsberg, 1996). On the other hand, Clarke *et al.* (1985), and Forsberg and Summerfelt (1992a) suggested that protein levels in the diet had no significant effect on ammonia excretion. Estimation of the relative share of protein catabolism in total energy used is possible by means of ammonia quotient (AQ). When AQ amounts to 0.33, all consumed oxygen is used for protein oxidation (Kutty, 1978). Our experiments revealed a significant, directly proportional relation between AQ and protein level in the diet. Mean AQ values ranged from 0.068 to 0.079, meaning that from 20.6% (diet B) to 23.9% (diet A) of oxygen consumed by the fish were used to oxidise proteins.

OC and AE values obtained in the experiments were similar to those obtained for *S. lucioperca* weighing 38.1 g (BW) and 16.0 cm long (TL), reared in a recirculation system in 22°C, for which mean OC and AE amounted to 243 mg O_2/kg/h and 12 mg TAN/kg/h, respectively (Zakes, 1998). Similar values of oxygen consumption and ammonia excretion were also found by Forsberg and Summerfelt (1992b) for the walleye, *Stizostedion vitreum* (Mitchill) of similar size (16.2–16.8 cm TL), reared in 20 and 25°C: 214 mg O_2/kg/h and 19 mg TAN/kg/h, and 294 mg O_2kg/h and 25 mg TAN/kg/h, respectively.

Requirements of fish for dietary proteins depend on the species and stage of ontogenic development (Murai, 1992; Jobling, 1994). Barrows (1987) (cit. according to Stettner *et al.*, 1992), and Barrows *et al.* (1988) found that optimal dietary levels of proteins for *S. vitreum* of body weight 14 and 50 g which guaranteed maximal growth rate of the fish were 51.0 and 42.3%, respectively. The use of diets containing different levels of proteins from 42.2 to 52.5% to feed *S. lucioperca* was not reflected in fish growth. The highest growth rate of pikeperch reared from 16.5 to 19.4 cm TL at water temperature of 22°C amounted to 1.0 mm/d and 0.83 g/d. For comparison, average daily increment of *S. vitreum,* reared from 12.4 to 16.8 cm TL in controlled conditions using artificial feeds, was 0.63 mm/d (Barrows *et al.*, 1988). Siegwarth and Summerfelt (1990) stated that for juvenile *S. vitreum* reared from 14.6 to 17.8 cm TL at 21°C this increment was 0.45 mm/d.

The use of diets differing in protein levels was not reflected in the level of body protein of pikeperch fry. The same conclusion was drawn by Reinitz and Austin (1980a), who reared juvenile *S. vitreum* on diets containing 34.3–57.0% protein. Protein content in fish body was 15.9–16.8% of wet weight, and the differences between the feeding variants were not significant. Neither in the case of rearing yellow perch, *Perca flavescens* (Mitchill), was there any effect of dietary protein levels on protein content in fish body (Reinitz and Austin, 1980b). Our studies revealed a noticeable effect of dietary fat levels on fat content in *S. luciperca* body. This relationship was also found in *S. vitreum* and *P. flavescens* (Reinitz and Austin, 1980a, b), as well as in other non-percid fish species (Reis *et al.*, 1989; Li and Lovell, 1992). Our own data, as well as those given by other authors suggest that dietary protein and fat levels determined body fat levels, but not so much protein content. This is also confirmed by the comparison of body composition of pikeperch reared on commercial feeds and that consuming natural food. Fat levels in fish eating commercial diets were 7 times higher than in those growing on natural food. An earlier study also showed that in the case of fish reared in controlled conditions and on artificial diet and zooplankton, the difference in body fat levels was 6.7-fold, while body protein concentrations differed by 5.4% only (Zakes and Demska-Zakes, 1998).

REFERENCES

Barrows F.T. (1987): Nutrient requirements of walleye (*Stizostedion vitreum vitrum*). Ph. D. Thesis, Iowa State University, Ames. 139 pp.

Barrows F.T., Sell J.L., Nickum J.G. (1988): Effects of dietary protein and energy levels on weight gains, body composition, and RNA-DNA ratios of fingerling walleye. Progress. Fish-Cult., *50*, 211–218.

Clarke E.R., Harman J.P, Forster J.R.M. (1985): Production of metabolic and waste products by intensively farmed rainbow trout, *Salmo gairdneri* Richardson. J. Fish Biol., *27*, 381–393.

Forsberg O.I. (1996): Ammonia excretion rates from post-smolt Atlantic salmon, *Salmo salar* L., in land-based farms. Aquacult. Res., *27*, 937–944.

Forsberg J.A., Summerfelt R.C. (1992a): Ammonia excretion by fingerling walleye fed two formulated diets. Progress. Fish-Cult., *54*, 45–48.

Forsberg J.A., Summerfelt R.C. (1992b): Effect of temperature on daily ammonia excretion of fingerling walleye. Aquaculture, *102*, 115–126.

Jobling M. (1994): Fish Bioenergetics. Chapman & Hall, London. 300 pp.

Jobling M., Davies P.S. (1980): Effects of feeding on metabolic rate, and the Specific Dynamic Action in plaice, *Pleuronectes platessa* L. J. Fish Biol., *16*, 629–638.

Kaushik S.J. (1980): Influence of nutritional status on the daily patterns of nitrogen excretion in the carp (*Cyprinus carpio* L.) and rainbow trout (*Salmo gairdneri* R.). Reprod., Nutr. Develop., *20*, 1751–1765.

Kutty M.N. (1978): Ammonia quotient in Sockeye Salmon (*Oncorhynchus nerka*). J. Fish. Res. Board of Canada, *35*, 1003–1005.

LeGrow S.M., Beamish W.H. (1986): Influence of dietary protein and lipid on apparent heat increment of rainbow trout, *Salmo gairdneri*. Can. J. Fish. Aqua. Sci., *43*, 19–25.

Li M., Lovell R.T. (1992): Growth, feed efficiency and body composition of second- and third-years channel catfish fed various concentrations of dietary protein to satiety in production ponds. Aquaculture, *103*, 153–163.

Murai T. (1992): Protein nutrition of rainbow trout. Aquaculture, *100*, 191–207.

Reinitz G., Austin R. (1980a): Practical diets for intensive culture of walleye. Progress. Fish-Cult., *42*, 212–214

Reinitz G., Austin R. (1980b): Experimental diets for intensive culture of yellow perch. Progress. Fish-Cult., *42*, 29–31.

Reis L.M., Reutebach E.M., Lovell R.T. (1989): Protein-to-energy ratios in production diets and growth, feed conversion and body composition of channel catfish, *Ictalurus punctatus*. Aquaculture, *77*, 21–27.

Schmidt-Nielsen K. (1994): Animal Physiology: Adaptation and environment. Cambridge University Press, Cambridge. 602 pp.

Siegwarth G.L., Summerfelt R.C. (1990): Growth comparison between fingerling walleye and walleye × sauger hybrids reared in intensive culture. Progress. Fish-Cult., *52*, 100–104.

Skulmowski J. (1974): Basic analysis. In: J. Skulmowski (ed.): Methods of Determination of Feed and their Quality (in Polish). PWRiL, Warsaw. 14–25 pp.

Stettner C.R., Summerfelt R C., Kuipers K.L. (1992): Evaluation of commercial feeds for rearing advanced fingerling walleye. In: Proc. Annual Conference of Southeast Association of Fish and Wildlife Agencies, *46*, 402–412.

Zakes Z. (1998): Effect of body weight on oxygen consumption and ammonia excretion by juvenile pikeperch, *Stizostedion lucioperca* (L.) (Percidae) reared in recirculating water system. FAO Fish. Rep., *580*, 32–33.

Zakes Z., Demska-Zakes K. (1998): Intensive rearing of juvenile *Stizostedion lucioperca* (Percidae) fed natural and artificial diets. Ital. J. Zool., *65*, 507–509.

Zakes Z., Karpinski A. (1999): Influence of water temperature on oxygen consumption and ammonia excretion of juvenile pikeperch, *Stizostedion lucioperca* (L.) reared in a recirculating system. Aquacul. Res., *30*, 109–114.

Received: 00–03–27
Accepted after corrections: 00–11–10

Corresponding Author:

Dr. Zdislav Zakes, The Stanislaw Sakowicz Inland Fisheries Institute, 10-719 Olsztyn-Kortowo, Oczapowskiego 10, Poland Tel. +48 89 524 01 71, fax +48 89 524 05 05, e-mail: irs@uwm.edu.pl

Age and growth of pike (*Esox lucius* L.) in irrigation canals of the East Slovakian Lowland

Vek a rast šťuky severnej (*Esox lucius* L.) v melioračných kanáloch Východoslovenskej nížiny

J. Koščo

Department of Ecology, Faculty of Human and Natural Sciences, University of Prešov, Slovak Republic

ABSTRACT: For age and growth analysis 131 pikes (*Esox lucius* L.) have been examined; out of them 111 were adults (48 males, 63 females) and 20 juveniles. The fish were divided into five age groups while group I comprised 43.5% of the fish. In view of the considerable size of age group 0 (30.5%), the canals showed to be an appropriate biotope for spawning. The shortage of older fish is probably caused by the unfavourable conditions during winter and after the peak of the vegetation period. In the lower parts of the irrigation canals the pike grew faster than in the middle and upper parts. The flow rate slows down growth; however, the danger of the rise of conditions lethally affecting the ichthyocenoses is decreased. The growth rate of pike in the irrigation canals at Hraň in the first year of life is approximately the same as in other localities; later, however, it slows down.

Keywords: environmental conditions; fishes; *Esox lucius* L.; irrigation canals; Central Europe

ABSTRAKT: Na analýzu veku a rastu šťuky bolo použitých 131 rýb, z toho 111 dospelých (48 samcov, 63 samíc) a 20 juvenilných jedincov. Ryby patrili do 5 vekových skupín, najpočetnejšia bola 1. veková skupina (43,5%). Vzhľadom na značné zastúpenie 0. vekovej skupiny (30,5%), kanály sú vhodným biotopom pre neres šťuky. Nedostatok starších rýb je pravdepodobne zapríčinený nepriaznivými podmienkami počas zimy a po vrchole vegetačného obdobia. V dolných úsekoch kanálov rástli šťuky rýchlejšie ako v stredných úsekoch. Prietočnosť v kanáloch spôsobuje pomalší rast, ale znižuje nebezpečenstvo vzniku podmienok pôsobiacich na ichtyocenózy letálne. V porovnaní s inými lokalitami rastie šťuka v hydromelioračných kanáloch pri Hrani v prvom roku približne rovnako rýchlo, v ďalších rokoch je jej rast pomalší.

Kľúčové slová: životné prostredie; ryby; Esox lucius L.; melioračný kanál; Stredná Európa

INTRODUCTION

Although large populations of pike live in the irrigation canals of the East Slovakian Lowland (ESL), little is known about their ecology in these biotopes. As age and growth are indices contributing to the true picture of the locality, the present work aimed at the analysis of the above factors in the given species. Sedlár (1971b) also analyzed the age and growth of pike; however the irrigation canals of the Nitra river catchment, where he carried out his research, are of a different nature. Literary data on fish biology in the canals of the ESL were only presented by Koščo (1988).

MATERIAL AND METHODS

Study site

The network of irrigation canals at Hraň (Fig. 1) consists of two main canals (K1, K2) emptying into the river Ondava through a common pumping station (PS), and having a double trapezoid profile of 10 m width and 1.5–2.0 m depth. K2 is longer than K1 (26 vs. 12 km). The canals K3 and K4 are shorter (2 vs. 4 km), narrower (6 vs. 8 m) and shallower (1.0 vs. 1.5 m), and both empty into K2. The lowest and highest slopes are seen in canal K1 (0.05%) and K2 (0.15%), respectively.

The bed of the canals consists of a mainly organic sediment of autochthonous origin; prevailingly black anaerobic mud can be found. The canals are overgrown with submerse and natant vegetation. Accompanying littoral trees are either sporadic or missing at all. The physical and chemical properties of canal water are given in Tab. 1. *Esox lucius* live there in a community together with *Rhodeus sericeus* (79.0%), *Rutilus rutilus* (5.6%), *Scardinius erythrophthalamus* (4.1%), *Perca fluviatilis* (2.7%), *Carassius carassius* (1.4%), *Misgurnus fossilis* (0.7%), *Tinca tinca* (0.7%), *Leucaspius delineatus* (0.6%), *Alburnus alburnus* (0.5%) and *Ictalurus nebulosus* (0.5%). (The relative abundance of *Esox lucius* was 4.2%).

The constructions of the canals system and the pumping station enable the migrations between river and canals during the most part of the year.

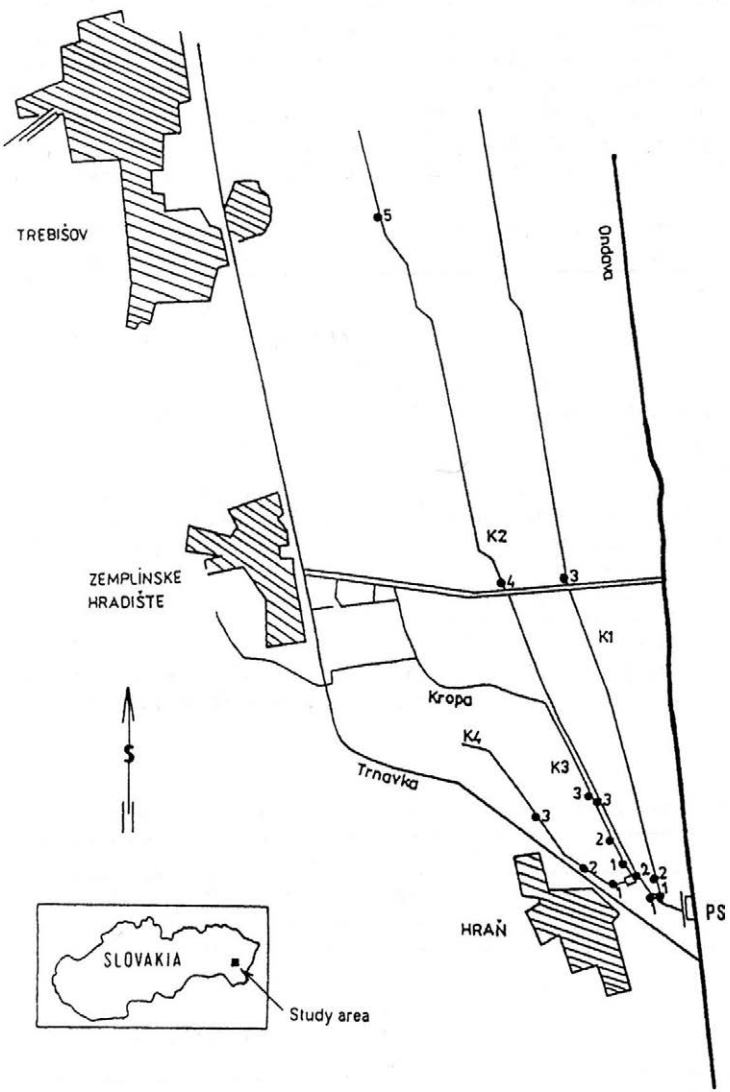

Figure 1. Sampling site localization in studied irrigation canals

Table 1. Physical and chemical properties of water in the irrigation canals at Hrań

Character (Unit)	K1 (1987)			K2 (1987–88)			K3 (1987-88)			K4 (1988)		
	Min.	Average	Max.	Min.	Average	Max.	Min.	Average	Max.	Min.	Average	Max.
Water temperature (°C)	0.2	13.8	26.9				0.3	13.0	27.0	1.5	15.8	27.2
pH	7.4	8.0	9.6				6.2	7.7	8.9	6.0	7.3	8.0
COD (mg/l)	6.80	11.20	15.20	6.12	7.56	9.00	4.20	9.40	15.20	9.00	12.80	25.40
DO (mg/l)	1.5	8.4	19.6				1.0	10.6	21.0	1.0	8.1	12.0
Conductivity (mS)	0.7	1.0	1.2				0.1	1.0	1.2	0.7	1.0	1.3
Turbidity	0.01	0.13	0.40				0.01	0.20	0.89	0.01	0.10	0.10
Hardness (mmol/l)	1.6	3.3	6.2	2.0	2.2	2.3	1.5	2.7	4.4	2.1	2.7	3.3
Ca^+ (mg/l)	39.1	111.0	238.9	12.0	63.4	84.3	36.1	84.2	171.4	48.7	78.7	108.2
Mg^+ (mg/l)	6.9	15.8	38.1	7.3	19.2	41.3	2.4	17.7	38.2	2.4	19.9	31.3
Cl^- (mg/l)	25.5	126.1	372.2	1.5	23.0	35.1	22.7	45.4	99.3	13.4	44.1	76.6
NH_4^- (mg/l)	0.001	0.260	0.542	0.420	0.540	0.700	0.020	0.440	1.900	0.130	0.710	2.580
NO_3^- (mg/l)	0.01	0.06	0.16	0.00	0.03	0.07	0.02	0.04	0.09	0.02	0.05	0.11
NO_4^- (mg/l)	0.70	5.83	35.00	0.20	2.25	4.25	0.40	5.03	22.90	0.32	2.98	6.40
PO_4^- (mg/l)	0.05	0.90	1.95	0.15	0.71	2.25	0.05	0.72	2.30	0.10	0.46	1.03
Alkalinity (mg/l)	3.1	3.5	4.0	3.5	3.7	3.8	1.9	4.4	5.7	1.7	4.2	5.8
Na^+ (mg/l)	36.0	53.1	102.0	19.2	26.8	43.5	25.0	45.5	78.0		28.5	
K^+ (mg/l)	5.5	12.7	25.5	5.4	5.6	7.5	6.5	15.9	30.0		27.5	

Material

Age and growth analysis have been made of 131 fish (111 adults – 48 males and 63 females, 20 juveniles), caught by means of seine (length 12 m, depth 3 m, mesh diameter 10 mm). In spring (April 20–22, 1988) fish were repeatedly caught in the lower, middle and upper parts of all canals (i.e. at 200, 700 and 1 200 m distance from the PS). The aim was to determine the differences between the indices under examination in dependence on the distance from the PS. In the course of 1988, fish were caught from the lower parts of the canals K2, K3 and K4 at monthly intervals (20 April, 17 May, 8 June, 30 June, 9 August, 28 September) in order to determine the differences between the individual canals.

The samples were preserved *in situ* with 4 – 6% formaline; measuring and weighing of the fish [total and standard lengths, total and body (after evisceration) weight, weight of the digestive tract and the gonads] were carried out in the laboratory.

Six representative scales were taken from the part above the lateral line in front of the dorsal fin. Age was determined from the dorsodiagonal lobe of the scales by means of the Zeiss Lesegerät projector at a magnification 17.5x.

Methods

The growth rate of the standard length was determined by the method of R. Lee (Holčík and Hensel 1972). As it

Table 2. Regression equations (GM) of the standard length and weight relation in pike from irrigation canals at Hrań (our own data)

Locality	Sex	Regression equation		
All canals	all fish	$\log w = -5.0900 + 3.0522 \log l$	$r = 0.9814$	$n = 131$
All canals	adult males	$\log w = -5.1597 + 3.0837 \log l$	$r = 0.9959$	$n = 48$
All canals	adult females	$\log w = -4.8899 + 2.9561 \log l$	$r = 0.9817$	$n = 63$
All canals	juveniles	$\log w = -5.0763 + 3.0462 \log l$	$r = 0.9947$	$n = 20$
K2L2	all fish	$\log w = -4.9132 + 2.9612 \log l$	$r = 0.9881$	$n = 48$
K3L1	all fish	$\log w = -5.0615 + 3.0391 \log l$	$r = 0.9987$	$n = 20$
K4L1	all fish	$\log w = -5.0930 + 3.0482 \log l$	$r = 0.9965$	$n = 32$

was impossible to determine directly the standard length of the fish at the time of the formation of scales, the correction value of 40 mm was taken over from the work of Oliva (1956). The values of weight increase were derived according to the GM regression equations of the relation between body length and weight. No significant differences were found between the length: weight relation in males and females as well as in samples from different canals (Tab. 2); thus for weight increase determination the following regression equation has been used:

$\log w = -5.09000 + 3.0522 \log l$, $r = 0.9814$, $n = 131$, representing the fish analyzed in this work.

The condition coefficient was calculated according to Clark; the fish were weighed after evisceration.

RESULTS

The standard length of fish (Sl) at capture ranged between 63 and 350 mm while their weight ranged between 2.7 and 532 g. In the Slovak Republic legally admittable size (Tl) is 500 mm. The standard length of the only fish that reached catching size (its Tl being 640 mm) was 562 mm (Sl). The mean standard length of the subsequent age groups in each sampling is given in Fig. 2.

The investigated fish belonged to five age groups, age groups I and 0 being the most numerous (43.5 and 30.5 %, respectively). The higher age groups II, III and IV comprised 23.7 , 1.5 and 0.8 % of the fish, respectively. During the peak of vegetation (July, August) only fish of age groups 0 and I could be observed in the canals; in August, however, only fish of the age group 0 were recorded in K4 while no fish at all were found in K3. The most favourable age structure was observed in K2.

At the time of the first capture (April 20–22) in most fish (86%) the annulus could already be observed. Out of the 7 fishes without a pronounced annulus two were males (aged 3 years) and 5 females (aged 2 years); of the latter one had not yet spawned and the other 4 had the rest of old eggs in their gonads.

On the basis of the back calculated body length and weight values, the growth rate of pike seemed to be slow, males growing faster than females (Tab. 3). The fish grew faster in the lower parts of the canals than in the middle and upper parts. Comparison of the growth rates of fish from the lower parts of K2, K3 and K4 revealed that in the first year of life pike in K3 and K4 grew faster but less of them survived. In the first year of life the growth rate of pike in K2 decreased; however, more of the older fish survived (Fig.2).

The highest condition coefficient was in all age groups in fish from K4 and lowest in those from K2. With growing length (age) the condition coefficient increased and reached its highest values in September.

As to the sex ratio, the number of males surpassed that of females at a ratio of 1.3 : 1.0. In spring, females prevailed in the lower parts while males prevailed in the upper parts of all canals (K2, K3, K4).

DISCUSSION

In the canals at Hraň pike reach low body weights and lengths. The lower mean standard length (Fig. 2) or even absence of fish during the peak of the vegetation period may coincide with the deterioration of the physical and chemical properties of canal water after overgrowth and subsequent extinction of large amounts of submerse vegeta-

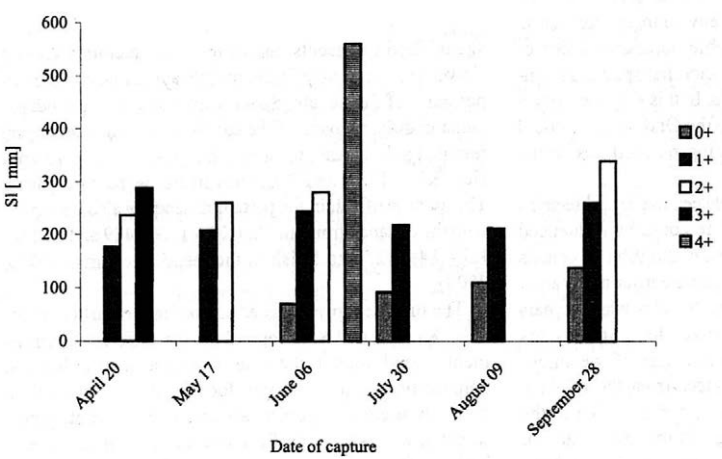

Figure 2. Mean standard length of particular age groups of pike from irrigation canals at Hraň at the time of netting

Table 3. Mean back calculated standard length (mm) and weight (g) of pike from the canals at Hraň

Sex	Age	Number	Standard length at capture	Weight at capture	Length/weight in particular years			
					1	2	3	4
Males	1+	27	195	92	159/52			
	2+	9	344	286	210/50	308/182		
	3+	2	288	279	148/221	221/123	276/256	
	total	38	235	148	170/50	292/171	276/256	
Females	1+	30	212	107	174/61			
	2+	22	235	159	138/198	198/106		
	4+	1	560	1 672	139/22	257/162	335/361	498/1 179
	total	53	228	158	159/49	201/109	335/361	498/1 179
All fish	1+	57	204	100	170/56			
	2+	31	255	196	159/38	230/128		
	3+	2	288	279	148/34	221/123	276/256	
	4+	1	560	1 672	139/22	257/162	335/361	498/1 179
	total	91	227	154	165/49	230/129	309/291	498/1 179

tion (e.g. at an amount of 7.0–7.5 kg/m^2 wet phytomass, the level of dissolved oxygen decreased, during the early morning hours to 0.43–0.59 mg/l).

The absence of larger specimens may be caused by increased mortality during the unfavourable winter period (findings of dead fish after the ice had thawed). Neither fishing pressure nor fish mortality can be excluded; according to Kipling (1984) the latter is one of the factors considerably limiting pike populations mainly those of larger specimens to the end of summer.

There is a relation between the mean standard length and the age structure of pike population in the canals. Most individuals belonged to age groups I and II (97.7%), which from the viewpoint of reproduction is particularly unfavourable. The most favourable age structure was recorded in canal K2; this can be explained by better winter survival conditions (higher water level) and the decreased danger of anaerobic environment formation (increased slope). The considerable numbers of fish of age group 0 (30.5%) point to the fact that spawning conditions in the canals are favourable. In this way the system of irrigation canals emptying into the Ondava river could substitute the shortage of spawning possibilities in the regulated river.

Growth differences recorded between males and females in the first and second years of life could be influenced by differences in the formation and maturation of gonads mainly if environmental impacts cause earlier maturation (1 year old males grow slower than females while 2 years old males grow faster). The data given for 3 and 4 years old fish are distorted by the low numbers of specimens and their capture at various distances from the PS (fish aged 3 years were caught in the upper parts while the 4 years old specimens were caught in the lower parts of the canals) (Tab. 4). According to our opinion, the shortage of food represents one of the main factors affecting slower growth of pike in the middle and mainly in the upper parts of the canals. Species diversity of the ichthyofauna in different parts of the canals (lower, middle, upper) revealed a decreasing tendency (e.g. K4 – 13, 6 and 5 species, K3 – 12, 8 and 2 species in the respective parts). The number of fish in the particular samples also decreased with the distance from the PS (K4 – 1 198, 439 and 64 fish; K3 – 849, 125 and 5 fish in the respective parts; Koščo, 2000).

Table 4. Back calculated mean standard length (mm) and weight (g) growth of pike from various parts of the canals at Hraň

Canal	Part	Length/weight in particular years			
		1	2	3	4
K1	lower	150/19	206/84		
K2	lower	171/50	252/158	335/366	498/1 184
	middle	159/48			
	upper	151/37	214/127	285/350	
K3	lower	180/60	234/131		
	middle	159/47	248/177		
	upper	158/46	228/137	266/218	
K4	lower	179/60	230/127		
	middle	141/31	202/85		
	upper	138/30	181/67		

The different growth rates of pike in the particular canals (K2, K3 and K4) depend on the different environmental conditions in the latter (water level, number and composition of macrophyte, food base...; Terek, 1999), given by their topography and the way of maintenance. In canal K2, where the flow rate in relation to slope is highest, the growth rate of pike in the first year of age

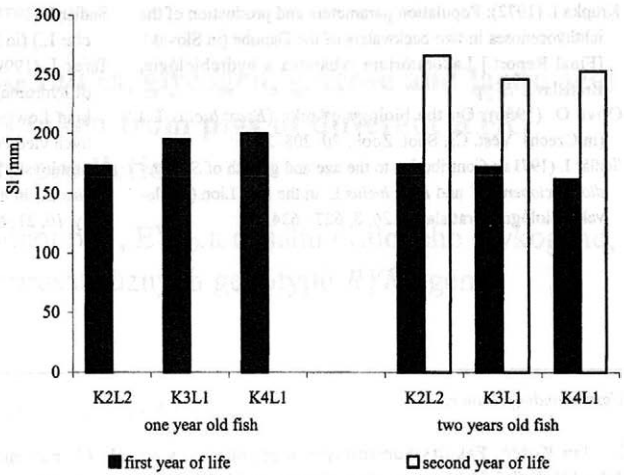

Figure 3. Back calculated growth rate of standard length in one and two years old pike from the lower parts of the irrigation canals at Hraň

Table 5. Growth of pike at different localities

Type of biotope	Locality (author)	Length/weight in particular years				
		1	2	3	4	5
Valley reservoirs	Oravská priehrada (Balon, 1965)	323/143	340/440	425/850	475/1 200	590/2 260
	Lipno (Vostradovský, 1977)	249/243	382/960	469/1 782	565/2 912	679/4 170
Backwaters of the Danube	Vlčie hrdlo (Krupka, 1972)	162/60	232/151	275/245		
	Rusovce (Krupka, 1972)	102/10	182/52	249/179	445/875	
	Lion (Sedlár, 1971b)	177/58	292/270	389/565	464/1 050	549/1 720
Branch of the Danube	Trstená n/O. (Černý,1992)	152/44	355/472	398/738	491/1 428	534/1 720
Irrigation canal	Hraň (this study)	165/49	230/129	309/291	498/1 179	

was slower as compared to the canals K3 and K4. More favourable winter conditions and lower shares of submerse and natant vegetation decrease the danger of overpopulation; this certainly affects the survival of older individuals in K2. Although the growth rate in K3 and K4 was higher, their populations were low and only a part of the smaller individuals survived (Fig. 3).

When compared to other localities the growth rate of pike in the canals of the Ondava river catchment is lower (Tab. 5). The fish in the canals at Hraň showed comparable growth rates only in the first year of life; later their growth slowed down. This coincides with data on the growth of *Rhodeus sericeus*, collected in the same biotopes (Koščo, 1988).

In agreement with Sedlár (1971a) it can be stated that growth variance in the canals does not reach that extent as observed in larger water bodies as growth conditions in the canals are less diverse.

REFERENCES

Balon E.K. (1965): Wachstum des Hechtes (*Esox lucius* L.) im Orava – Stausee. Z. Fisch. Hilfswiss, *13*, 113–158.

Černý J. (1992): Age, growth and index of production of pike (*Esox lucius* L.) from the Danube arm Trstená na Ostrove (in Slovak). Biológia (Bratislava), *47*, 153–162.

Holčík J., Hensel K. (1972): Handbook of Ichthyology (in Slovak). Obzor, Bratislava. 220 pp.

Kipling C. (1984): A study of perch and pike (*Esox lucius*) in Windermere from 1941 to 1982. J. Const. Int. Explor. Mer., *41*, 259–267.

Koščo J. (1988): Age and growth of *Rhodeus sericeus amarus* (Bloch 1783) in irrigation canals of the Ondava river basin at Hraň (in Slovak). Biológia (Bratislava), *43*, 927– 934.

Koščo J. (2000): Ichthyofauna of the irrigation canals at Hraň (Ondava river basin). Tiscia.

Krupka I. (1972): Population parameters and production of the ichthyocenoses in two backwaters of the Danube (in Slovak). [Final Report.] Laboratórium rybárstva a hydrobiológie, Bratislava. 73 pp.

Oliva O. (1956): On the biology of pike (*Esox lucius* L.) (in Czech). Věst. Čs. Spol. Zool., *20*, 208–223.

Sedlár I. (1971a): Contribution to the age and growth of *Stizostedion lucioperca* L. and *Esox lucius* L. in the lake Lion (in Slovak). Biológia (Bratislava), *26*, 8, 627– 634.

Sedlár I. (1971b): A case of very slow growth of pike (*Esox lucius* L.) (in Slovak). Poľnohospodárstvo, *17*, 232– 235.

Terek J. (1999): Structure and function of biotic components of hydromelioration canals in the Hraň Region, East-Slovakian Lowland (in Slovak). Fakulta humanitných a prírodných vied Prešovskej univerzity v Prešove, Prešov. 110 pp.

Vostradovský J. (1977): The age and growth of pike (*Esox lucius* L.) in the artificial reservoir Lipno. Práce VÚRH, Vodňany, *10*, 21–46.

Received: 00–04–25
Accepted after corrections: 00–11–10

Corresponding Author:

Dr. Jan Koščo, Fakulta humanitných a prírodných vied, ul. 17, novembra 1, 081 16 Prešov, Slovenská republika
Tel. +421 91 773 32 31, e-mail: kosco@unipo.sk

Usefulness of pH_1 and EC_{50} values, glycogen, glucose and lactic acid content in biopsy samples taken from pigs of different *RYR1* genotypes for meat quality prediction

Odhad kvality masa podle hodnot pH_1, EV_{50} a obsahu svalového glykogenu, glukózy a kyseliny mléčné u prasat různých genotypů *RYR1* genu

L. Křenková, T. Urban, J. Kuciel

Mendel University of Agriculture and Forestry, Brno, Czech Republic

ABSTRACT: The aim of this study was to compare the values of pH_1, EC_{50}, muscle glycogen, glucose and lactic acid content in the commercial hybrid pigs with different *RYR1* genotypes and the prediction of meat quality from biopsy samples of *Musculus longissimus lumborum et thoracis* taken *in vivo*, approximately at 80 kg live weight. The results showed that *RYR1* genotypes influence the values of meat quality (pH_1 and EC_{50}) both after slaughter and from biopsies, carried out about 5 weeks before slaughter. The occurrence of PSE meat ranged in biopsy samples according to pH from 10.16 to 14.58% but after slaughter from 9.47 to 54.35%. The correlation coefficients between pH ($r = 0.39$; $P \leq 0.05$) and EC_{50} ($r = 0.23$; $P \leq 0.05$) from biopsies and after slaughter were very low. The levels of muscle glycogen, glucose and lactic acid did not differentiate between the *RYR1* genotypes. There was a high variability in measured traits between and within the groups of *RYR1* genotypes. The results indicated that the values of pH_1, EC_{50}, glycogen, glucose and lactic acid in biopsy samples were not useful for prediction of meat quality after slaughter.

Keywords: pig; pH_1; electric conductivity; muscle glycogen, glucose, lactic acid content

ABSTRAKT: Cílem práce bylo srovnání hodnot pH_1, elektrické vodivosti (EV_{50}), obsahu svalového glykogenu, glukózy a kyseliny mléčné u prasat různého genotypu *RYR1* genu a odhad kvality masa podle měření uvedených ukazatelů v bioptických vzorcích *Musculus longissimus lumborum et thoracis* odebraných *in vivo* v živé hmotnosti asi 80 kg. Genotyp *RYR1* genu ovlivnil kvalitu masa (pH_1 a EV_{50}) jak po porážce, tak při biopsii, tzn. asi pět týdnů před porážkou. Výskyt PSE masa podle pH_1 kolísal v bioptických vzorcích od 10,16 do 14,58 %, ale po porážce od 9,47 do 54,35 %. Korelační koeficienty hodnot pH_1 ($r = 0,39$; $P \leq 0,05$) a EV_{50} ($r = 0,23$; $P \leq 0,05$) mezi měřeními při biopsii *in vivo* a po porážce byly velmi nízké. V obsahu svalového glykogenu, glukózy a kyseliny mléčné nebyly rozdíly mezi *RYR1* genotypy statisticky průkazné. Mezi skupinami i uvnitř skupin genotypů *RYR1* genu byla zjištěna značná variabilita těchto ukazatelů. Jejich využití k predikci kvality masa se jeví podle našich výsledků sporné.

Klíčová slova: prase; pH_1; elektrická vodivost; svalový glykogen, glukóza, kyselina mléčná

INTRODUCTION

A set of biochemical and biophysical processes, so-called meat ageing, takes place in the muscle tissue after slaughter. Especially, degradation of the energy components of the muscles (glycogen and ATP) occurs in this period. The accumulation of lactic acid in the course of anaerobic glycogenolysis after slaughter is responsible for a decrease in pH, which has a great impact on the final meat quality (Leach *et al.*, 1996; Fisher and Mellett 1997; Karlsson *et al.*, 1997; Rothschild and Ruvinsky, 1998). Pale, soft and exudative (PSE) meat differs from normal meat in more intensive muscle glycogen degradation, accumulation of lactic acid, rapid decrease in pH

Supported by the Ministry of Education of the Czech Republic (Project No. MSM 432100001).

within 45 min after slaughter below the value 5.6, considerable decrease in ATP, higher muscle temperature and rigidity and in rapid development of *rigor mortis*. The rapid decrease in pH is frequently used to objectify the identification of PSE meat (Ingr, 1996).

The incidence of PSE is influenced both by genetic predispositions (the point mutation in the *RYR1* gene results in the porcine stress syndrome, which is related to PSE meat, Fujii *et al.*, 1991) and environmental factors (physiological conditions, reaction to transport, stress before slaughter, slaughter technology, manipulation with carcasses, etc., Stupka and Šprysl, 1998).

The aim of this study was to compare the values of pH_1, EC_{50}, muscle glycogen, glucose and lactic acid content in the commercial hybrid pigs with different *RYR1* genotypes and prediction of meat quality from biopsy samples of *Musculus longissimus lumborum et thoracis* taken *in vivo*.

MATERIAL AND METHODS

Experimental animals

A total number of 145 commercial hybrid pigs from Large White x Landrace sows x Large White$_{\text{sire line}}$ or Belgic Landrace or hybrid Piétrain x Large White$_{\text{sire line}}$ and Czech Meat Pig x Belgic Landrace boars were studied. The parents of experimental animals were selected on the basis of their *RYR1* genotypes (mothers *Nn* x fathers *nn*), biopsies of *Musculus longissimus lumborum et thoracis in vivo* (MLLT) and meat quality traits measurements were carried out in their progeny. The genotypes of *RYR1* gene were determined according to Brenig and Brem (1992) using PCR-RFLP methods.

Meat quality evaluation

Pigs were weaned at the age of 28–35 days and fattened from 25–30 kg live weight. Meat quality was evaluated in biopsy samples (0.50–0.80 g) taken at the live weight of approximately 80 kg (B) from the MLLT. The biopsy samples were taken by an efficient non-stress spring-loaded biopsy instrument PPB-3 (Kováč *et al.*, 1993) on the right side just below the last rib 50 mm laterally from the spine by means of a needle 9 x 70 mm in diameter. The simulation of the after-slaughter processes was made by means of incubation of the muscle samples for 50 min 39°C in the thermoblock. The meat quality traits were determined on the basis of: pH_1 value measured using a digital pH-meter Gryf 209 S with glass electrodes Gryf PCL 223 in biopsy samples (BpH_1); the values of electric conductivity (EC_{50}) were measured using a digital conductometer PMV micro (Biotech, Slovak Republic) with the electrode, in the biopsy samples (BEC_{50}). We supposed that the rate of the anaerobic processes in the incubated muscle tissue from live pigs was the same as that after slaughter (Kováč *et al.*, 1992; Mlynek, 1995). The rest of the samples was frozen in liquid nitrogen immediately after biopsy for an analysis of glycogen, glucose and lactic acid contents. The pigs with the live weight of ca. 110 kg were transported to the abattoir and slaughtered by electric stunning. The samples of MLLT were taken also 1 hour after slaughter for evaluation of meat quality ($pH_1 - SpH_1$, electric conductivity – SEC_{50}). These meat quality classes were distinguished according to pH_1 and EC_{50} values (Stupka *et al.*, 1993):

pH_1	EC_{50}	meat quality class
≥ 5.81	≤ 4.99	normal
5.61–5.80	5.00–7.99	tendency to PSE
≤ 5.60	≥ 8.00	PSE

Determination of glycogen, glucose and lactic acid contents in biopsy samples of MLLT

Glycogen, glucose and lactic acid contents (in μmol/g tissue) were determined in frozen biopsy samples by absorbance measurements. The glycogen concentration (GLYC) was determined according to Dreiling *et al.* (1987). In this method glycogen reacts with iodine solution in the presence of potassium iodide. The solution is then saturated with $CaCl_2$. The glucose content (GLUC) was determined using a diagnostic kit Bio-La-Test Oxochrom glucose (Lachema, Czech Republic). The glucose was oxidized by oxygen and by catalysis with glucoseoxidase to hydrogen peroxide and gluconate. The hydrogen peroxide was determined after the reaction with substituted phenol and 4-aminoantipyrin catalysed peroxidase. The lactic acid concentration (LAC) was determined after conversion to the acetaldehyde (using sulphuric acid). The acetaldehyde was determined after the reaction with p-hydroxydiphenyl in the presence of copper ions by spectrophotometric measurement (Davídek, 1981).

Statistical methods

The basic statistical characteristics, Student's *T*-test of significance of differences between the mean values and Pearson's correlation coefficients were used.

The General Linear Model (SAS, 1988) was used for the estimation of relations between the *RYR1* genotypes and variability of meat quality traits in pigs. The model

$$y_{ijklm} = \mu + RYR_i + H_j + S_k + O_l + b.\text{age}_{ijklm} + e_{ijklm}$$

included genetic ($RYR1_i - RYR1$ genotypes, H_j – hybrid combination, S_k – sex) and non-genetic factors (O_l – the season of measurements and $b.\text{age}_{ijklm}$ – regression on the age at measurement).

RESULTS AND DISCUSSION

Highly significant differences were observed between *RYR1* genotypes *Nn* and *nn* in meat quality traits pH_1 and EC_{50} measured by biopsy *in vivo* (at ca. 80 kg live weight) and after slaughter (Tab. 1).

The recessive homozygous animals had highly significantly lower average BpH_1 values and higher average BEC_{50} values; they were characterised by meat quality with tendency to PSE, compared with heterozygous genotypes which had higher average BpH_1 values and lower average BEC_{50} values with a characteristic of normal meat quality. Pigs with *nn* genotype had highly significantly lower average SpH_1 values (measured after slaughter) than pigs with *Nn* genotype. In the literature there are many studies confirming the high meat variability of heterozygous animals. According to some results, the *Nn* pigs had meat quality comparable with pigs of *NN* genotype (Eikelenboom *et al.*, 1980 – cit. Cheah *et al.*, 1995), intermediate meat quality, in the middle of both homozygous genotypes (Murray and Jones, 1994) or meat quality comparable with recessive homozygous animals (Pommier and Houde, 1993). Nyström and Andersson (1993), De Smet *et al.* (1998) did not find any differences between *nn* and *Nn* genotypes. On the contrary, Przybylski *et al.* (1996) did not find any differences in pH_1 value between *Nn* and *NN* genotypes, the difference was observed between both homozygous animals only.

The recessive homozygous genotypes in our experiment achieved highly significantly higher average SEC_{50} values measured after slaughter (Tab. 1), they had PSE meat according to this value. Heterozygous animals had the meat with tendency to PSE according to SEC_{50} value. According to Stupka *et al.* (1993), electric conductivity is an indicator making it possible to evaluate meat quality more exactly.

The data were divided into three meat quality classes (Tab. 2) according to limiting values of pH_1 a EC_{50} and the differences were calculated between the average values of meat quality traits according to *RYR1* genotype in these classes.

According to pH_1 and EC_{50} values in biopsy samples in the PSE meat quality class (Tab. 2), the heterozygous animals achieved significantly lower BEC_{50} values than the pigs with *nn* genotype. We did not find any significant differences between the *RYR1* genotypes in the BpH_1 values in all meat quality classes (Tab. 2). We can partly compare our results with those published by Cheah *et al.* (1993). They observed lower pH_1 values of biopsy samples in the halothane-positive (stress-susceptible) pigs compared to halothane-negative ones. Henckel *et al.* (1992) found significant differences between *NN* and *Nn*, *nn* genotypes in pH_1 values of biopsy samples. The homozygous *NN* had higher pH_1 values than those of *Nn* or *nn* genotype. No differences in pH_1 between the heterozygous and recessive homozygous genotypes were observed.

A highly significantly lower SEV_{50} value was also observed in the heterozygous animals with the PSE meat quality class compared to the recessive homozygous animals in the same meat quality class (Tab. 2). Differences between the genotypes in SpH_1 values were insignificant in all meat quality classes. In the analysed population, there were pigs with normal meat, tendency to PSE and PSE meat (Tab. 2) in both genotypes. The occurrence of the PSE meat ranged (according to BpH_1 and BEC_{50} values

Table 1. Meat quality traits of the *RYR1* genotypes

Traits	*Nn*			*nn*		
	n	*LSM*	SE	*n*	*LSM*	SE
In vivo						
BpH_1	97	**5.97^A**	**0.11**	48	**5.73^A**	**0.11**
BEC_{50}	97	**4.75^A**	**0.68**	48	**6.49^A**	**0.70**
GLYC (μmol/g)	56	51.09	9.33	32	41.25	10.49
GLUC (μmol/g)	23	8.40	0.80	20	8.33	0.81
LAC (μmol/g)	23	160.26	25.47	20	185.31	26.54
After slaughter						
SpH_1	95	**6.12^A**	**0.10**	46	**5.79^A**	**0.11**
SEC_{50}	95	**6.40^A**	**0.71**	46	**8.32^A**	**0.75**

Note:
Values in the rows with the same letters show significant differences: $^aP \leq 0.05$; $^AP \leq 0.01$

Table 2. Meat quality classes determined according to pH_1 in the different *RYR1* genotypes

Meat quality		*RYR1* genotype		$(\bar{x} \pm t.s_{\bar{x}})$	
		%	*Nn*	%	*nn*
BpH_1			*n* = 97		*n* = 48
PSE	≤ 5.6	13.40	5.46 ± 0.03	10.41	5.41 ± 0.05
Tendency	5.61–5.8	11.34	5.70 ± 0.01	22.92	5.69 ± 0.02
Norm.	≥ 5.81	75.26	6.19 ± 0.02	66.67	6.08 ± 0.06
BEC_{50}			*n* = 97		*n* = 48
PSE	≥ 8.00	10.16	**8.56 ± 0.20^a**	14.58	**9.29 ± 0.15^a**
Tendency	5.00–7.99	47.42	6.05 ± 0.09	62.50	6.34 ± 0.90
Norm.	≤ 4.99	42.42	3.63 ± 0.17	22.92	3.89 ± 1.48
SpH_1			*n* = 95		*n* = 46
PSE	≤ 5.6	9.47	5.52 ± 0.02	45.65	5.52 ± 0.01
Tendency	5.61–5.8	12.63	5.69 ± 0.05	19.57	5.73 ± 0.02
Norm.	≥ 5.81	77.90	6.25 ± 0.22	34.78	6.16 ± 0.05
SEC_{50}			*n* = 95		*n* = 46
PSE	≥ 8.00	20.00	**9.26 ± 0.17^A**	54.35	**9.88 ± 0.12^A**
Tendency	5.00–7.99	38.95	6.14 ± 0.12	23.91	6.60 ± 0.21
Norm.	≤ 4.99	41.05	4.16 ± 0.12	21.74	4.02 ± 0.22

Note:
Values in the rows with the same letters show significant differences: $^aP \leq 0.05$; $^AP \leq 0.01$

determined in biopsy samples) from 10% to 14% (Tab. 2). The increasing number of *nn* pigs with PSE meat was observed according to SpH_1 and SEC_{50} values after slaughter; according to SpH_1 values, 46% and according to SEC_{50} values 54% recessive homozygous pigs had the PSE meat after slaughter (Tab. 2). (There was PSE meat from 10% according to SpH_1 to 20% according to SEC_{50} in the heterozygous genotypes.) 10% of heterozygous genotypes had PSE meat according to SpH_1, and 20% heterozygous genotypes had the same defect according to SEC_{50}.

As compared with the occurrence of PSE meat in biopsy samples (according to BpH_1 – 10.41%, Tab. 2) in the recessive homozygous animals, there was the increasing number of recessive homozygous genotypes with PSE meat after slaughter (according to SpH_1 – 45.65%). The negative effects of pre-slaughter handling on the meat quality were reduced to the minimum, but it was still possible that these effects represented a sufficient stress factor for stress-susceptible animals, i.e. those with *nn* genotype of *RYR1* gene. This assumption can explain an increasing number of the recessive homozygous animals with PSE meat after slaughter. Neither can we exclude the effects of various stressors influencing animals in the interval from biopsy to slaughter. Contrary to our observations, Cheah *et al.* (1995) found PSE meat in 56.7% of pigs with heterozygous genotypes on the basis of measurements of 5 meat quality traits in biopsy samples of MLLT.

We did not observe any differences in glycogen, glucose and lactic acid contents in biopsy samples between the *RYR1* genotypes (Tab. 1).

The animals were classified according to pH_1 and EC_{50} values into three meat quality classes (normal, tendency to PSE and PSE (Tab. 3). Pigs of *nn* genotypes had a significantly lower glycogen content (Tab. 3) in the PSE meat quality class determined in the biopsy samples taken at 80 kg live weight compared with other meat quality classes.

The same but statistically insignificant tendency was also observed in the glycogen content in the meat quality classes determined according to pH_1 and EC_{50} values after slaughter. A lower glycogen content in the PSE meat of the recessive homozygous animals could indicate more intensive anaerobic glycogenolysis in these pigs. Our results showed that *RYR1* genotype influenced pH_1 and EC_{50} values both *post mortem* and at 80 kg live weight (i.e. ca. 5 weeks before slaughter). Our results also suggested that a decrease in pH_1 in *nn* genotypes is a consequence of different rate of muscle glycogen degradation (Tab. 3) rather than of different glycogen contents (Tab. 1). With respect to a low number of analysed samples it will be necessary to confirm our results in other experiments.

We also found higher glucose and lactic acid contents in recessive homozygous genotypes in the PSE meat quality class as compared with normal meat and meat tendency to PSE; however these differences were insignificant (Tabs. 4 and 5). Przybylski and Kocwin-Podsiadla (1996) did not observe any significant effects of the *RYR1* genotype on glycogen content in Landrace pigs, but they found a significantly higher lactic acid content in *Nn* animals as compared with pigs of *NN* genotype. According to Esseen-Gustavson and Fjelkner-Modig (1985): cit. Henckel *et al.* (1992) the Hampshire breed, which is virtually free from the halothane gene, displays higher glycogen contents compared with the Landrace and Yorkshire with the higher frequency of *n* allele.

Different results in the Landrace and Large White breeds were reported by Przybylski *et al.* (1996). There were significant differences between *NN* and *Nn*, *nn* genotypes in the glycogen content in biopsy samples of MLLT taken at 60 kg live weight. Dominant homozygous genotypes had the higher glycogen level as compared with other genotypes.

We supposed that the measurements of glycogen, glucose and lactic acid content in combination with measurements of pH_1 and EC_{50} would help to explain meat quality variability within and between the groups of *RYR1* genotypes. However, the present results are preliminary only, and our suppositions are not confirmed. In addition there

Table 3. The phenotype variability of glycogen content (μmol/g) in the meat quality classes (determined according to pH_1 and EC_{50}) in the different *RYR1* genotypes

Meat quality		*RYR1* genotype		$(\bar{x} \pm t.s_x)$	
		%	*Nn*	%	*nn*
BpH_1			*n* = 56		*n* = 32
PSE	≤ 5.6	3.03	41.70 ± 12.05	5.88	**20.67 ± 0.12**[a]
Tendency	5.61–5.8	4.55	35.05 ± 11.62	17.65	**54.33 ± 10.62**[a]
Norm.	≥ 5.81	92.42	49.88 ± 4.31	76.47	**46.45 ± 5.43**[b]
BEC_{50}			*n* = 56		*n* = 32
PSE	≥ 8.00	35.71	40.65 ± 4.22	11.12	**22.17 ± 5 .64**[ab]
Tendency	5.00–7.99	29.59	33.93 ± 4.14	13.88	**47.00 ± 12.78**[b]
Norm.	≤ 4.99	34.69	58.88 ± 6.29	75.00	**54.63 ± 4.29**[a]
SpH_1			*n* = 56		*n* = 32
PSE	≤ 5.6	16.92	46.12 ± 7.91	58.82	41.44 ± 6.06
Tendency	5.61–5.8	15.38	40.88 ± 7.33	14.71	50.45 ± 12.9
Norm.	≥ 5.81	67.70	49.17 ± 5.47	26.47	54.89 ± 8.97
SEC_{50}			*n* = 56		*n* = 32
PSE	≥ 8.00	54.84	44.86 ± 5.87	64.70	40.87 ± 5.33
Tendency	5.00–7.99	20.97	39.71 ± 6.6	17.65	51.66 ± 12.10
Norm.	≤ 4.99	24.19	51.46 ± 9.30	17.65	61.00 ± 12.10

Note:
Values in the columns with the same letters show significant differences: $^aP \leq 0.05$; $^AP \leq 0.01$

Table 4. The phenotype variability of glucose content (μmol/g) in the meat quality classes (determined according to pH_1 and EC_{50}) in the different *RYR1* genotypes

Meat quality		*RYR1* genotype	($\bar{x} \pm t.s_{\bar{x}}$)		
		%	*Nn*	%	*nn*
BpH_1			*n* = 23		*n* = 20
PSE	≤ 5.6	–	–	9.52	8.91 ± 1.40
Tendency	5.61–5.8	3.85	7.17 ± 0.00	19.05	8.43 ± 0.17
Norm.	≥ 5.81	96.15	8.54 ± 0.31	71.43	8.14 ± 0.42
BEC_{50}			*n* = 23		*n* = 20
PSE	≥ 8.00	11.54	9.11 ± 0.83	9.52	9.54 ± 0.75
Tendency	5.00–7.99	19.23	8.98 ± 0.50	23.81	8.81 ± 0.43
Norm.	≤ 4.99	69.23	8.26 ± 0.40	66.67	8.41 ± 0.48
SpH_1			*n* = 23		*n* = 20
PSE	≤ 5.6	15.38	8.79 ± 0.56	57.14	8.88 ± 0.51
Tendency	5.61–5.8	7.70	7.05 ± 0.84	19.05	8.76 ± 0.63
Norm.	≥ 5.81	76.92	8.46 ± 0.37	23.81	8.67 ± 0.38
SEC_{50}			*n* = 23		*n* = 20
PSE	≥ 8.00	15.38	8.78 ± 0.73	14.29	8.91 ± 0.51
Tendency	5.00–7.99	65.39	8.48 ± 0.31	23.81	8.66 ± 0.82
Norm.	≤ 4.99	19.23	8.17 ± 1.17	61.90	8.72 ± 0.91

Table 5. The phenotype variability of lactic acid content (μmol/g) in the meat quality classes (determined according to pH_1 and EC_{50}) in the different *RYR1* genotypes

Meat quality		*RYR1* genotype	($\bar{x} \pm t.s_{\bar{x}}$)		
		%	*Nn*	%	*nn*
BpH_1			*n* = 23		*n* = 20
PSE	≤ 5.6	–	–	9.52	182.07 ± 14.57
Tendency	5.61–5.8	3.85	175.83 ± 39.21	19.05	165.49 ± 29.48
Norm.	≥ 5.81	96.15	158.97 ± 12.06	71.43	176.91 ± 13.61
BEC_{50}			*n* = 23		*n* = 20
PSE	≥ 8.00	11.54	176.72 ± 20.49	9.52	187.54 ± 27.16
Tendency	5.00–7.99	19.23	163.45 ± 20.93	23.81	162.40 ± 14.26
Norm.	≤ 4.99	69.23	138.11 ± 14.45	66.67	175.23 ± 22.00
SpH_1			*n* = 23		*n* = 20
PSE	≤ 5.6	15.38	171.49 ± 15.76	57.14	200.17 ± 16.51
Tendency	5.61–5.8	7.70	153.73 ± 41.98	19.05	162.97 ± 18.31
Norm.	≥ 5.81	76.92	134.77 ± 13.90	23.81	185.41 ± 16.45
SEC_{50}			*n* = 23		*n* = 20
PSE	≥ 8.00	15.38	171.22 ± 18.09	14.29	203.69 ± 14.94
Tendency	5.00–7.99	65.39	167.62 ± 14.53	23.81	177.91 ± 22.17
Norm.	≤ 4.99	19.23	154.33 ± 28.22	61.90	167.62 ± 19.31

was a high variability in these traits between and within the groups of *RYR1* genotypes.

Several significant but low or medium correlations were found between the pH_1 values from biopsy samples and after slaughter (0.39; $P < 0.05$); between EC_{50} values from biopsy samples and after slaughter (0.23; $P < 0.05$). Higher correlations were found between pH_1 and EC_{50} at biopsy and after slaughter measurements [–0.56; $P < 0.01$; –0.77; $P < 0.001$ (Tab. 6)]. The glycogen concentration was highly significantly correlated with the electric conductivity of biopsy samples (–0.32; $P < 0.01$) and lactic acid concentration with glucose content (0.44; $P < 0.05$).

Lahučký *et al.* (1982) found a highly significant correlation between the glycogen content and pH_1 of biopsy samples; however these findings were not confirmed in our study. There are differences in the absolute pH_1 values of biopsy samples and after-slaughter values. Based on this fact and low correlation coefficients we came to a conclusion that the biopsy test of pH_1 in pigs is questionable for the estimation of meat quality. pH_1 values are probably the trait that dynamically changes during ontogenesis. Therefore it is necessary to measure the values after slaughter to improve meat quality evaluation. Regarding these facts we recommend to select other methods of measuring of meat quality traits when we want to come to a final solution of this problem (R-value, WHC, etc.). For example Lahučký *et al.* (1997) and Cheah *et al.* (1993) found the high correlation coefficients between *in vivo* (biopsy) and after-slaughter measurements of fluid volume, R-value

Table 6. Correlation coefficients between meat quality traits

Trait	BpH_1	BEC_{50}	GLYC	GLUC	LAC	SpH_1	SEC_{50}
BpH_1	–	**–0.56****	–0.05	–0.02	–0.11	**0.39***	–0.17
BEC_{50}		–	**–0.32****	–0.05	–0.11	**–0.36*****	**0.23***
GLYC			–	0.04	0.16	0.13	–0.08
GLUC				–	**0.44***	–0.10	–0.008
LAC					–	0.04	–0.14
SpH_1						–	**–0.77*****
SEC_{50}							–

$^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$

and pH_1. These authors supported the suitability of biopsy prediction tests for the differentiation of animals with PSE meat.

REFERENCES

Brenig B. , Brem G. (1992): Molecular cloning and analysis of the porcine "halothane" gene. Arch. Tierzucht, *35*, 129–135.

Cheah K.S. , Cheah A.M. , Lahucky R. , Mojto J. , Kovac L. (1993): Prediction of meat quality in live pigs using stress-susceptible and stress-resistant animals. Meat Sci., *34*, 179–190.

Cheah A.M., Cheah K.S. , Krausgrill D.I. (1995): Variations in meat quality in live halothane heterozygotes identified by biopsy samples of *M. longissimus dorsi*. Meat Sci., *39*, 293–300.

Davídek J. (1981): Laboratorní příručka analýzy potravin. SNTL, ALFA, Praha. 395–396.

De Smet S., Bloemen H., Van de Voorde G., Spincemaille G., Berckmans D. (1998): Meat and carcass quality in two pig lines of different stress-susceptibility genotype and their crosses. Anim. Sci., *66*, 441–447.

Dreiling C.E., Brown D.E., Casale L., Kelly L.L. (1987): Muscle glycogen: Comparison of iodine binding and enzyme digestion assays and application to meat samples. Meat Sci., *20*, 167–177.

Fisher P., Mellett F.D. (1997): Halothane genotype and pork production. 1. Growth, carcass and meat quality characteristics. South Afr. J. Anim. Sci., *27*, 22–26.

Fujii J., Otsu K., Zorzato F., DeLeon S., Khanna V.K., Weiler J.E., O'Brien P.J., MacLennan D.H. (1991): Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science, *253*, 448–451.

Henckel P., Jorgensen P.F., Jensen P. (1992): Glycogen content, buffering capacity and resting pH in live muscles of pigs of different halothane genotypes (a pilot project). Meat Sci., *32*, 131–138.

Ingr I. (1996): Technologie masa. 1. vyd. MZLU Brno. 61–64.

Karlsson A., Henckel P., Petersen J.S., Andersson M. (1997): Early estimation of ultimate pig meat quality. In: 48th Annual Meeting of the EAAP, 25–28th August, Vienna, Austria, P 628.

Kováč L., Lahučký R., Mlynek J. (1992): Stress sensitivity and meat quality evaluation by biopsy method. In: Proc. 38th ICOMST, August 23–28, 1992, Clermont-Ferrand, France, Vol. 2, 1267–1270.

Kováč L , Mlynek J., Lahučký R., Eliáš J. (1993): Tlaková biopsia – nové možnosti odberu tukového a svalového tkaniva od hospodárskych zvierat. Veterinárstvi, *43*, 106– 107.

Lahučký R., Fischer K., Augustini Ch. (1982): Zur Vorhersage der Fleisch-beschaffenheit am lebenden Schwein mit Hilfe der Schussbiopsie. Fleischwirtschaft, *62*, 1323–1326.

Lahučký R., Christian L.L., Kováč L., Stalder K.J., Bauerová M. (1997): Meat quality assessed ante- and after slaughter by different ryanodine receptor gene status of pigs. Meat Sci., *47*, 277–285.

Leach L.M., Ellis M., Sutton D.S., McKeith F.K., Wilson E.R. (1996): The growth performance, carcass characteristics, and meat quality of halothane carrier and negative pigs. J. Anim. Sci., *74*, 934–943.

Mlynek J. (1995): Vplyv záťažových situácií na kvalitu mäsa ošípaných. Acta Zootechn. Univ. Agric., Nitra.

Murray A.C., Jones S.D.M. (1994): The effect of mixing, feed restriction and genotype with respect to stress susceptibility on pork carcass and meat quality. Can. J. Anim. Sci., *74*, 587–594.

Nyström P.E. , Andersson K. (1993): Halothane gene effects on reproduction, production and organ weights in pigs. Acta Agric. Scand., Sect. A, Anim. Sci., *43*, 201–206.

Pommier S.A., Houde A. (1993): Effect of the genotype for malignant hyperthermia as determined by a restriction endonuclease assay on the quality characteristic of commercial pork loins. J. Anim. Sci., *71*, 420–425.

Przybylski W., Kocwin-Podsiadla M. (1996): Glycolytic potential and lactate level was measured *in vivo* and also meat quality post mortem of pigs with $HAL^{N}HAL^{n}$ and HAL^{N}-HAL^{N} genotypes. In Conf. Current Problems of Genetics, Health, Growth and Production of Pigs, 6.–7. 2. 1996, České Budějovice, 87–89.

Przybylski W., Kocwin-Podsiadla M., Kaczorek S., Kuryl J., Krzecio E. (1996): Effect of the *RYR1* gene on the carcass composition and meat quality of Polish Landrace pigs. In: Conf. Current Problems of Genetics, Health, Growth and Production of Pigs, 6.–7. 2. 1996, České Budějovice, 84–86.

Rothschild M. F., Ruvinsky A. (1998): The Genetics of the Pig. CAB International, ISBN 0 85199 229 3.

SAS (1988): SAS/STAT. User's Guide (Release 6.03) SAS Inst. Inc. Cary, N. C.

Stupka R., Šprysl M. (1998): Stres a kvalita masa. <http://www.czu.cz/fakulty/af/kchpd/scripta/stres.html>

Stupka R., Pour M., Šprysl M., Císař M. (1993): Využití metody střelné biopsie a elektrické vodivosti ke stanovení kvality vepřového masa. Živoč. Výr., *37*, 369–376.

Received: 00–05–17
Accepted after corrections: 00–11–10

Corresponding Author:

Ing. Leona Křenková, PhD., Veterinární a farmaceutická univerzita, Palackého 1-3, 612 42 Brno, Česká republika
Tel. +420 5 41 56 23 26, e-mail: leak@email.cz

Pokyny pro autory příspěvků do vědeckého časopisu Czech Journal of Animal Science

Časopis uveřejňuje původní experimentální práce, krátká sdělení, přehledy, studie, informace a recenze. Práce jsou uveřejňovány v angličtině.

Autor je plně odpovědný za původnost práce a za její věcnou, jazykovou a formální správnost. K práci musí být přiloženo prohlášení autora o tom, že práce nebyla publikována jinde.

Práci určenou k uveřejnění dodají autoři s anglickým a českým (slovenským) abstraktem. Autoři jsou povinni zajistit kvalitní překlad do angličtiny. Rukopisy se všemi přílohami se dodávají ve dvou vyhotoveních a na disketě. V průvodním dopisu je třeba uvést editor použitý ke zpracování rukopisu na PC.

EXPERIMENTÁLNÍ PRÁCE by měla sestávat z těchto částí: titulní strana, abstrakt, klíčová slova, úvod, materiál a metody, výsledky, diskuse, poděkování, literatura, tabulky a obrázky (grafy) s výstižným popisem. Rozsah práce by neměl přesáhnout 15 stran včetně tabulek, obrázků atd.

KRÁTKÉ SDĚLENÍ by mělo obsahovat tyto části: titulní strana, abstrakt, klíčová slova, hlavní text, poděkování, literatura, tabulky a obrázky s popisem. Rozsah práce by neměl přesáhnout 4 strany.

PŘEHLEDNÝ ČLÁNEK (STUDIE, REVIEW) obsahuje souhrn poznatků dané problematiky. Rozsah článku by neměl být větší než 20 rukopisných stran a zpracovaná literatura by měla být z období posledních 20 let. K článku je třeba připojit krátký abstrakt v angličtině a češtině (slovenštině).

Titulní strana

Název práce (titul) musí výstižně informovat o zaměření práce a neměl by přesáhnout 85 úhozů. Nesmí obsahovat zkratky a nadbytečná slova (hodnocení, studium, popis, stručné výsledky, předběžné informace apod.). Jsou vyloučeny podtituly článků. Uvádí se v angličtině a v češtině nebo slovenštině.

Autoři se uvádějí zkratkou křestního jména a příjmením (u žen je možné použít plné křestní jméno).

Pracoviště autorů se uvádí v angličtině v oficiální verzi. Pokud jsou autoři z více pracovišť, uvádí se u jejich jména číslo odpovídající stejnému číslu v seznamu pracovišť.

Abstrakt musí obsahovat podstatné údaje o metodickém přístupu k řešení problému, výstižně popsané dosažené výsledky a jejich statistickou významnost a stručné a jednoznačné závěry, které z nich autoři vyvozují. V abstraktu se necitují žádné publikace a nediskutují výsledky.

Klíčová slova musí co nejlépe charakterizovat studovaný problém.

ÚVOD by měl informovat o aktuálním stavu výzkumu v daném tématu a o cíli práce. Citovanými publikacemi se dokládá stav současných poznatků, z nichž autoři vycházejí, nikoliv vše, co již bylo uveřejněno. Citace uvedené v textu musí souhlasit s údaji v seznamu literatury. Uváděny by měly být pouze citace z lektorovaných periodik.

MATERIÁL A METODY musí poskytnout jasný popis použitých:

- Strojů, přístrojů a zařízení, chemikálií, diagnostických souprav apod. (typ, zdroj nebo výrobce, země původu) a způsob jejich použití.
- Zvířat/rostlin (přesná definice druhu, počtu a zdrojů, jejich rozdělení do pokusných a kontrolních skupin, způsob a podmínky chovu/pěstování, hodnocení stavu, odběr vzorků a jejich uchovávání do zpracování).
- Metod (podrobný popis nebo citace dostupné práce, ve které je popis metody); je nepřípustné citovat práci, která pouze odkazuje na metodu, ale neuvádí její popis. Případné modifikace citovaných postupů musí být jednoznačně popsány. Pokud metody neprováděli autoři práce, musí být uvedeno, kdo dodal výsledky (jméno a pracoviště).
- Způsobů vyhodnocení výsledků, statistických metod a použitých programů.

VÝSLEDKY musí být zpracovány přehledně a pokud možno vyjádřeny graficky nebo v tabulkách. Není přípustná dokumentace stejných výsledků jak v tabulkách, tak ještě také v grafech; nejsou přípustné prostorové (trojrozměrné) grafy. Statistické vyhodnocení musí mít logické zdůvodnění a dokonalé zpracování. Výsledky by měly obsahovat údaje, které umožní jejich ověření (např. při hodnocení významnosti rozdílu dvou průměrů je třeba uvést průměr, počet stanovení a směrodatnou odchylku). Statisticky významné rozdíly musí být v tabulkách nebo v grafech vyjádřeny jednoznačně a přehledně. V práci nelze uvádět výsledky získané postupem, který není popsán nebo citován v metodice.

DISKUSE může být spojena s popisem výsledků nebo uvedena v samostatné části, ale v tom případě se v diskusi popis výsledků nesmí opakovat. Diskuse má jednoznačně vyjádřit srovnání dosažených výsledků s dosud známými poznatky, aby bylo zřejmé, co je ve výsledcích zcela novým poznatkem, v čem se dosažené výsledky liší od nálezů jiných autorů nebo v čem se s nimi shodují. Diskuse má zdůraznit význam výsledků a upozornit na nově otevřené otázky a na potřebu jejich řešení. V posledním odstavci diskuse by mělo být uvedeno, zda bylo dosaženo cíle vytčeného v úvodu a jaké z toho autoři vyvozují závěry.

Poděkování vyjadřuje uznání autorů za pomoc s interpretací výsledků, za posouzení práce a přispění k jejímu zpracování, za finanční nebo jinou podporu, za technickou spolupráci, za poskytnutí materiálů nebo důležitých informací, za provedené

vyšetření bez aktivní tvůrčí spoluúčasti na řešení, za překlad nebo jazykovou úpravu apod. Poděkování je také cestou k odlišení autorů od spolupracovníků nebo jiných účastníků, kteří k práci přispěli svým námětem, radou, poskytnutím metodik, resp. pokusného materiálu z jiných experimentů, či umožnili využití přístrojů apod., ale nezúčastnili se sami aktivně a tvůrčím způsobem řešení popisovaného problému.

LITERATURA (seznam citovaných prací) musí zahrnovat všechny práce citované v textu (v případě citací více prací z jednoho roku se v textu i v seznamu literatury odlišují písmeny a, b, c...). Pokud má práce více autorů, uvádějí se v seznamu prací všichni (v textu však jen první z nich s dodatkem *et al.* a rokem vydání). Autoři se uvádějí malými písmeny, křestní jména iniciálami, v závorce rok vydání, za dvojtečkou název citované práce, oficiální zkratka časopisu, svazek, stránkový rozsah.

Literatura by měla sestávat z lektorovaných periodik. Není žádoucí, aby byly citovány výsledky z referátů přednesených na konferencích, z výzkumných zpráv, z disertačních a habilitačních spisů, z učebnic a monografií bez popisu nebo citací experimentálních prací, z populárního nebo z denního tisku a z obtížně dostupných (pro zahraničí zcela nedostupných) zdrojů. Jen výjimečně lze v diskusi připustit citaci nepublikovaného názoru nebo výsledku s uvedením autora a s poznámkou (nepublikováno) nebo (osobní sdělení). Tato citace se neuvádí v seznamu citovaných prací.

Příklad citací v seznamu (zkratky časopisů se uvádějí podle Science Citation Index nebo Current Contents):

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

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

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

Práce v monografiích a ve sbornících se uvádějí podle následujícího vzoru:

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

V seznamu literatury nelze uvádět práce, které nejsou citovány v textu.

Příklad citace v textu: ...popsal Brown (1995), ...popsala řada autorů (Brown, 1995; Green and Grey, 1996; Reed *et al.*, 1998)...

Tabulky a obrázky se dodávají zvlášť a na všechny musí být odkaz v práci. Všechny ilustrativní materiály by měly mít kvalitu vhodnou pro tisk. Je nežádoucí, aby velikost grafu a velikost popisů v něm byly v nepoměru (velký graf a drobný popis nebo naopak). Fotografie i grafy jsou v textu uváděny jako obrázky a jsou číslovány průběžně. Každý obrázek musí mít stručný a výstižný popis.

Kontaktní adresa jednoho z autorů, na kterého může být zasílána korespondence, je uváděna v češtině nebo slovenštině (popř. v angličtině u zahraničních autorů) na konci práce. Kromě poštovní adresy obsahuje čísla telefonu a faxu a e-mail adresu.

Další požadavky

Rukopis práce se dodává vytištěný běžnými fonty ve velikosti 12. Další technické požadavky (zpracování obrázků a možnost uveřejnění fotografií, doporučené textové editory, formáty digitalizovaných obrázků apod.) si lze vyžádat v redakci. Rukopis včetně obrázků na disketě musí být vždy doplněn i současným odesláním vytištěné verze v požadovaném počtu dvou výtisků. Textový soubor se na disketu ukládá bez dělení slov a zarovnání bloků. Rukopis musí být psán s širokým okrajem, dvojitými mezerami mezi řádky a na papíru formátu A4. Jestliže jsou grafy vytvořeny v programu EXCEL (všechny stejným způsobem), je potřeba je dodat s tabulkou, z níž byl příslušný graf vytvořen.

Zkratky a symboly používané v práci je nutné při jejich prvním uvedení vysvětlit. Používané jednotky musí odpovídat soustavě měrových jednotek SI.

Lektorský posudek autoři pečlivě prostudují a práci podle připomínek co nejdříve upraví a vrátí redakci. Současně s upravenou prací se vrací i původní rukopis s doprovodným dopisem, v němž je uveden seznam provedených úprav, případně důvod proč s připomínkami lektora autoři nesouhlasí. Autoři mají právo odmítnout stanovisko lektora, musí však svůj názor písemně zdůvodnit. Redakce pečlivě zvažuje stanovisko autorů, nemusí však jejich námitkám proti připomínkám lektorů vyhovět.

Korektury se provádějí do dvou dnů s použitím běžných korektorských značek (možno vyžádat v redakci). Provedení korektury se označuje slovem IMPRIMATUR v pravém horním rohu první strany, datem a podpisem. Při korekturách nelze provádět větší změny textu nebo změny, které mění význam sdělení nebo stanoviska autorů v rukopisu přijatém k uveřejnění.

Separáty: Autor obdrží zdarma 10 separátních výtisků práce.