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INFLUENCE OF RAPE SEEDS THERMAL PROCESSING ON THE LIMITATION OF LYPOLYSIS PROCESSES AND BIOHYDROGENATION OF UNSATURATED FATTY ACIDS IN RUMEN (PART I)

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Abstract. The aim of the present work was to assess the efficiency of thermal treatment of rape seeds in the reduction of its fat lypolysis in rumen and unsaturated fatty acids biohydrogenation. The studies were conducted using *in sacco* method with polyamide sacs on 6 rams of Merino breed with simple fistula of rumen. The protection of caked rape seeds involved its thermal processing at temperature 120°C for 30 minutes. Raw and thermally processed rape seeds were incubated in rumen for 2, 4, 8, 16 and 24 hours and thermal decomposition of air dry mass, raw fat, fatty acids with various degree of saturation and particular fatty acids were studied. On the basis of the obtained results, it was found that thermal processing of rape seeds resulted in significant reduction of occurring in rumen lypolysis of their fat and biohydrogenation of unsaturated fatty acids (mono- as well as polyunsaturated). Moreover, it was found that in the seeds that were processed with thermal method, monounsaturated fatty acids were protected better than polyunsaturated ones.

Key words: rumen, rape seeds, thermal protection, fat and unsaturated fatty acids

INTRODUCTION

Fats of animal origin contain mainly saturated fatty acids that favor the development of various diseases referred as civilization ones [Ziemlański and Budzyńska-Topolowska 1991].

In the case of monogastric animals, the advantageous modification of fatty acids profile (increase in the content of unsaturated fatty acids and decrease in saturated ones) can be obtained by an enrichment of their diet with vegetable fat rich in unsaturated fatty acids [Ajuyah et al. 1991, Chachułowa et al. 1997, Morgan et al. 1992].

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However, the modification of fatty acids composition in ruminants is more difficult, due to a specific structure of their digestive tract and microflora present in their stomachs. Exceeding the fat level in diet of ruminants above 5–7%, disadvantageously influences metabolism and digestion in rumen [Grummer 1988]. Moreover, reducing environment of rumen causes that the majority of unsaturated fatty acids are subject to hydrogenation process in rumen [Mills *et al.* 1970].

One of the methods to avoid reduction of the quantity of fat in the diet for ruminants as well as protection of unsaturated fatty acids from transformation in rumen, is the application of fat in the protected form.

The aim of the present work was to assess the effect of thermal processing of caked rape seeds on the reduction of the processes of their fat lypolysis and biohydrogenation of unsaturated fatty acids in rumen.

MATERIAL AND METHODS

In the preliminary studies on rape seeds of “00” species, the analyses of the content of dry matter, total protein and raw fat [Ładoński and Gospodarek 1986] as well as determination of fatty acids profile were conducted.

In order to perform chromatographic determination, caked samples of rape seeds were esterified twice with Folch mixture (chloroform in methanol in the ratio 2:1). Lipid fraction of fat isolated in this way, after filtration through anhydrous sodium sulfate, was subject to hydrolysis with 0.5 mol/dm³ of KOH and esterification with 14% BF₃ in the presence of methanol. In the next stage, methyl esters of fatty acids in the quantity 1 µl were transferred to gas chromatograph PU4410 (Philips) with flame-ionic detector (FID) and 105 m long capillary column Rtx-2330 at Industrial Chemistry Research Institute, Warsaw, Poland.

In the experimental part at the laboratory of Institute of Animal Breeding, Wrocław University of Environmental and Life Sciences, caked rape seeds of “00” species were processed thermally. The protective agent was temperature of 120°C for 30 minutes. In the prepared seeds, the content of the dry matter, total protein and raw fat and the composition of fatty acids was determined again.

In the second part of the study, decomposition of air dry matter (PSM) and raw fat (TS) as well as mono- and polyunsaturated fatty acids in both raw and prepared seeds was assessed. The studies were conducted with *in sacco* method with the application of polyamide sacs on 6 rams of Polish Merino breed with simple fistula to the rumen. In order to perform the study, 4 g of raw and protected with thermal method caked rape seeds were introduced to the rumen and incubated for 2, 4, 8, 16 and 24 hours. The experiment was conducted in the following system: 1 sac x 6 animals, that is in 6 repetitions for each type of the material studied and incubation time, according to the procedure given by Ørskov and McDonald [1979]. Due to insufficient quantity of the material studied that remained after incubation, in order to perform further analyses, the samples were simultaneously incubated and raw fat (in two repetitions) and the fatty acids composition were determined. Extraction of fat and chromatographic analyses of fatty acids composition were carried out at Industrial Chemistry Research Institute, Warsaw, Poland.

To assess the significance of differences between analysed parameters of PSM, TS and fatty acids, SAS (1996) statistical software was used.

Due to very low levels of the analyzed parameters (obtained by longer incubation times in the rumen), all the results presented in tables were 100-times multiplied.

RESULTS

Table 1 presents the chemical composition of rape seeds and their fatty acids profile.

The content of total protein in rape seeds was 19%, and of raw fat 46 %. About 89% of all fatty acids in rape seeds consisted of unsaturated fatty acids, and 11% of saturated fatty acids. In the group of unsaturated fatty acids, monounsaturated fatty acids dominated – 71%, mainly oleic acid C_{18:1}.

Thermal processing that rape seeds were subject to did not influence significantly the content of dry matter, total protein and raw fat as well as fatty acids composition (data not published).

Table 1. The composition of raw rape seeds [%]

Tabela 1. Skład nasion rzepaku

Specification Wyszczególnienie	Content [%] Zawartość
Dry matter – Sucha masa	94.80
Protein – Białko	19.20
Raw fat – Tłuszcz surowy	46.10
Fatty acids – Kwasy tłuszczowe:	
C _{8:0}	0.10
C _{10:0}	0.10
C _{12:0}	0.10
C _{14:0}	1.00
C _{16:0}	5.60
C _{16:1}	0.40
C _{17:0}	0.30
C _{18:0}	2.40
C _{18:1}	59.50
C _{18:2}	18.40
C _{18:3}	6.80
C _{20:0}	0.70
C _{20:1}	3.00
C _{22:0}	0.60
C _{22:1}	0.90

The results concerning the rate of decomposition of raw and thermally processed rape seeds and lypolysis of their fat in rumen are presented in table 2.

Decomposition of fat in raw rape seeds after 2 hours of incubation was 67%; after 4 hours – 77%; after 8 hours – 90%, however after 16 and 24 hours almost 100%. In the case of processed seeds, decomposition of fat was as follows: 44%; 56%; 74%; 85% and 89%. The differences detected in the rate of lypolysis of raw fats and thermally prepared rape seeds were statistically highly significant.

Table 2. Decomposition of dry matter and fat in raw and thermally processed rape seeds in rumen of sheep [g]

Tabela 2. Rozkład w żwaczu suchej masy oraz tłuszczu w surowych i poddanych obróbce termicznej nasionach rzepaku

Specification Wyszczególnienie	Type of seeds Rodzaj nasion	Time of seeds incubation in rumen [hours] Czas inkubacji nasion w żwaczu [godz.]					
		0	2	4	8	16	24
Dry matter Sucha masa	Raw – Surowe	400.0	150.9 ^A	103.5 ^A	47.6 ^A	21.2 ^A	11.7 ^A
	Protected – Chronione	400.0	240.0 ^A	194.5 ^B	166.0 ^B	107.9 ^B	96.8 ^B
Raw fat	Raw – Surowe	184.6	60.6 ^A	42.4 ^A	19.3 ^A	1.7 ^A	0.9 ^A
Thuszcz surowy	Protected – Chronione	188.2	104.6 ^B	82.7 ^B	49.8 ^B	27.3 ^B	21.4 ^B

A, B – difference significant on the level of $P \leq 0.01$ – A, B – różnice istotne na poziomie $P \leq 0,01$

Table 3 presents the results concerning the degree of unsaturated fatty acids decomposition.

After 2, 4, 8, 16 and 24 hours of incubation, decomposition of unsaturated fatty acids in raw and processed rape seeds was 67 and 45%; 77 and 56%; 90 and 74%; 99 and 86%, 99 and 89%, respectively (all differences were statistically highly significant).

Table 3. Decomposition of fatty acids with different degree of saturation of raw and processed thermally rape seeds in rumen [g]

Tabela 3. Rozkład w żwaczu kwasów tłuszczowych o różnym stopniu nasycenia w surowych i poddanych termicznej obróbce nasionach rzepaku

Type of seeds Rodzaj nasion	Incubation time [hours] Czas inkubacji [godz.]	Fatty acids [g] Kwasy tłuszczowe		
		Unsaturated Nienasycone	Monounsaturated Jednonienasycone	Polyunsaturated Wielonienasycone
Raw / Surowe	0	163.6	117.1	46,5
Protected / Chronione		167.3	118.0	49,2
Raw / Surowe	2	53.6 ^A	40.9 ^A	12,7 ^A
Protected / Chronione		91.9 ^B	70.6 ^B	21,2 ^B
Raw / Surowe	4	37.5 ^A	28.7 ^A	8,6 ^A
Protected / Chronione		73.5 ^B	56.6 ^B	16,9 ^B
Raw / Surowe	8	16.4 ^A	13.2 ^A	3,2 ^A
Protected / Chronione		43.1 ^B	34.0 ^B	9,1 ^B
Raw / Surowe	16	1.4 ^A	1.7 ^A	0,1 ^A
Protected / Chronione		23.2 ^B	20.2 ^B	3,0 ^B
Raw / Surowe	24	1.3 ^A	1.2 ^A	0,08 ^A
Protected / Chronione		18.9 ^B	16.8 ^B	2,1 ^B

A, B – differences significant on the level of $P \leq 0.01$ – A, B – różnice istotne na poziomie $P \leq 0,01$

The process of rape seeds heating also reduced the rate of single unsaturated fatty acids biohydrogenation in rumen. In the case of seeds that were prepared with thermal method, the process of biohydrogenation of oleic $C_{18:1}$ (fig. 1), linoleic $C_{18:2}$ (fig. 2) and linolenic $C_{18:3}$ (Fig. 3) acids was significantly slower than raw seeds.

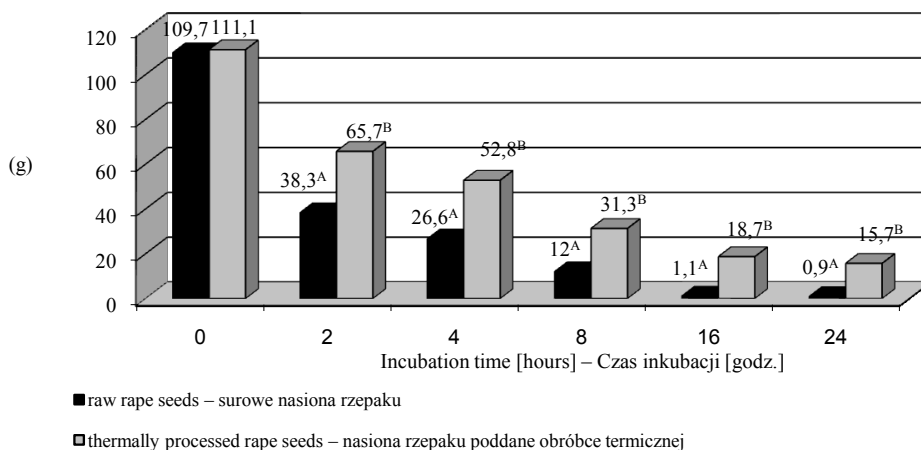


Fig. 1. Decomposition of oleic acid $C_{18:1}$ in raw and thermally processed rape seeds after incubation in the rumen [g]

Rys. 1. Rozkład kwasu oleinowego $C_{18:1}$ w surowych i poddanych obróbce termicznej nasionach rzepaku po inkubacji w żwaczu

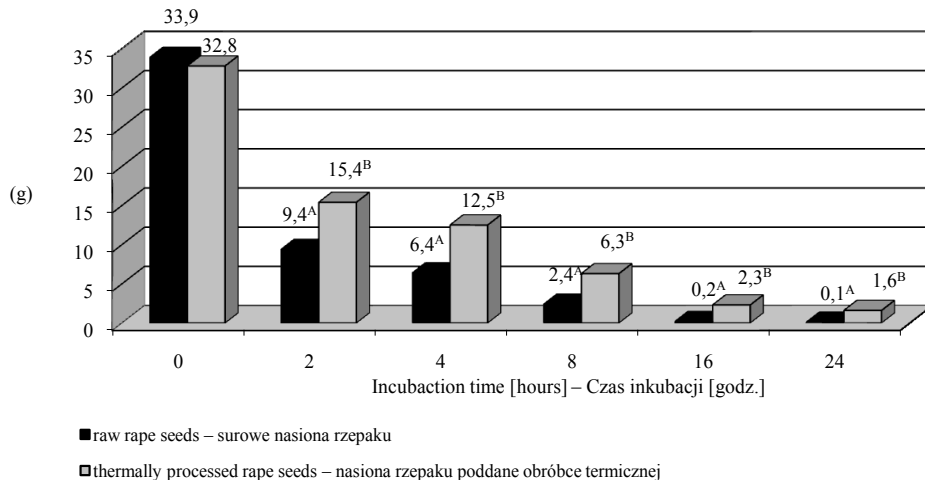


Fig. 2. Decomposition of linoleic acid $C_{18:2}$ in raw and thermally processed rape seeds after incubation in the rumen [g]

Rys. 2. Rozkład kwasu linolowego $C_{18:2}$ w surowych i poddanych obróbce termicznej nasionach rzepaku po inkubacji w żwaczu

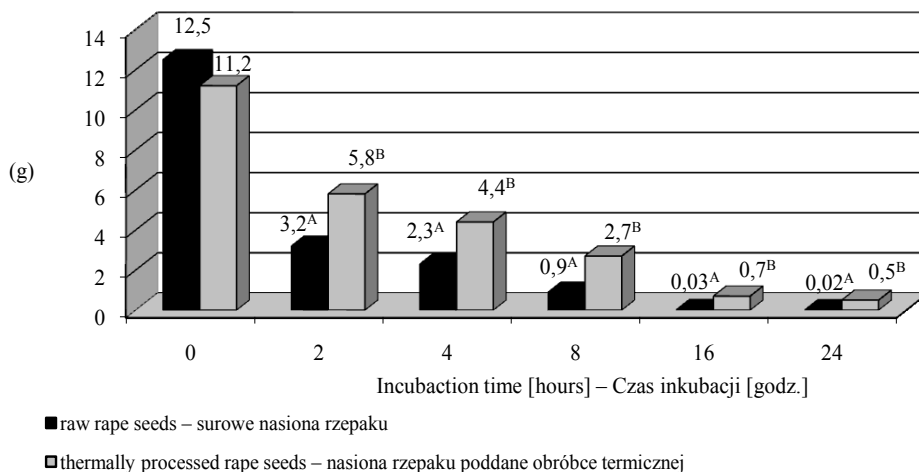


Fig. 3. Decomposition of linoleic acid C18:3 in raw and processed thermally rape seeds after incubation in the rumen (g)

Rys. 3. Rozkład kwasu linolenowego C18:3 w surowych i poddanych obróbce termicznej nasionach rzepaku po inkubacji w żwaczu (g)

DISCUSSION

In the present study, the effect of rape seeds thermal processing on the reduction of some transformations that occur in their fat in rumen of ruminants was investigated.

The obtained results enabled to assess that the process of rape seeds thermal processing significantly reduced the rate and the extent of lypolysis of fat and biohydrogenation of unsaturated fatty acids in the rumen.

The application of high temperature (120°C for 30 min) resulted in partial denaturation of protein of rape seeds which decreased their susceptibility to proteolysis and deamination in the rumen. Due to the increase of the resistance of protein integuments on microbiological proteolysis of fat molecules in non-disintegrated form, the seeds passed through the rumen (pH 6–7) into stomach, where more acidic environment (pH 2–3) caused hydrolysis of protein integument and release of fat. For this reason, enzymatic decomposition of fat to fatty acids occurred not in the rumen but in small intestine.

The efficiency of thermal method in the protection of fat from other seeds of oil plants was also demonstrated by other authors [Kowalski et al. 1995, Popiołek 1999].

Interesting results for the protection of fat in plants were also obtained by hydrothermal processing that involved heating with water vapor [Kozłowska et al. 1987]. The efficient way of protection of fat is also providing its in the form of calcium or magnesium salts [Kraszewski et al. 1993, Sklan et al. 1990]. In the case of liquid plant oils, a method of protection is also encapsulation in hardened gelatin capsules resistant to decomposition in the rumen [Edmondson et al. 1974, Patkowska-Sokoła and Bodkowski 1995, Patkowska-Sokoła et al. 1994].

CONCLUSIONS

1. Thermal processing of rape seeds significantly reduced the rate and extent of fat lypolysis and biohydrogenation of unsaturated fatty acids in rumen of sheep.
2. In the seeds that were subject to thermal processing, monounsaturated and polyunsaturated fatty acids were protected to the larger extent against the process of biohydrogenation.
3. Due to heating treatment of rape seeds, a part of fatty acids, i.e. oleic C_{18:1}, linoleic C_{18:2} and linolenic C_{18:3}, passed through the rumen to stomach in an unchanged form.

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WPLYW TERMICZNEJ OBRÓBKI NASION RZEPAKU NA ZAHAMOWANIE PROCESU LIPOLIZY TŁUSZCZU I BIOUWODOROWANIA NIENASYCONYCH KWASÓW TŁUSZCZOWYCH W ŻWACZU (CZEŚĆ I)

Streszczenie. Celem pracy była ocena wpływu zabiegu ogrzewania nasion rzepaku na ograniczenie procesów lipolizy tłuszczu oraz biouwodorowania nienasyconych kwasów tłuszczowych w żwaczu. Badania przeprowadzono metodą *in sacco*, z użyciem poliamidowych woreczków, na 6 tryczkach rasy merynos z przetokami prostymi do żwacza. Zabieg termicznej ochrony ześrutowanych nasion rzepaku polegał na ich ogrzewaniu w temp. 120°C przez 30 min. Surowe i preparowane termiczną metodą nasiona rzepaku inkubowano w żwaczu w czasie 2, 4, 8, 16 oraz 24 godzin i oznaczano rozkład: powietrznie suchej masy, tłuszczu surowego, grup kwasów tłuszczowych o różnym stopniu nasycenia oraz poszczególnych kwasów tłuszczowych. Na podstawie uzyskanych wyników stwierdzono, że termiczna obróbka nasion rzepaku statystycznie istotnie ograniczyła proces lipolizy tłuszczu w żwaczu oraz biouwodorowanie nienasyconych kwasów tłuszczowych (zarówno jedno-, jak i wielonienasyconych). Ponadto zaobserwowano, że kwasy tłuszczowe jednonienasycone w większym stopniu chronione były przed biouwodorowaniem niż wielonienasycone.

Słowa kluczowe: żwacz, nasiona rzepaku, ochrona termiczna, tłuszcz i nienasycone kwasy tłuszczowe

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MACRO ANATOMICAL INVESTIGATION OF THE CERVICOTHORACIC GANGLION IN NEWBORN PERSIAN CATS

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Summary. The cervicothoracic or stellate ganglion belongs to the cervical part of sympathetic system. In animals it is formed by the combination of the caudal cervical ganglion with the first and often second thoracic ganglion. The study was conducted on 20 newborn Persian cats [7 males and 13 females] coming from the 58th day of gestation. The research has shown that the cervicothoracic ganglion is formed from the fusion of three or four ganglia: the caudal cervical ganglion [GCca], first thoracic ganglion [GTh1], second thoracic ganglion [GTh2], third thoracic ganglion [GTh3]. It is situated in C7-8 -Th1-3 segment. It is characterized by variable location relative to the cervical and thoracic segment of the vertebral column [skeletotopy] and various shape [oval, oblong, triangular, oblong with a distinct narrowing]. The morphometry of the examined cervicothoracic ganglion proved that the longest cervicothoracic ganglion is found on the left side in females [= 3.016 mm], the shortest on the right side also in females [= 2.790 mm]. The widest ganglion is found in males on the right side [= 1.758 mm], the narrowest in females on the left side [= 1.418 mm].

Key words: Persian cats, newborn, autonomic nervous system, sympathetic nervous system, stellate ganglion

INTRODUCTION

The cervicothoracic or stellate ganglion (*ganglion cervicothoracicum s. ganglion stellatum*) belongs to the cervical part of the sympathetic system (*pars cervicalis sympathic*) [Sobociński 1985, Marciniak and Ziółkowski 1992]. In animals it originates from the fusion of the caudal cervical ganglion (*ganglion cervicale caudale*) with the first thoracic ganglion (*ganglion thoracicum I*) and often also with the second thoracic ganglion (*ganglion thoracicum II*) [Sobociński 1985, Kuder 2002]. It is situated between the transverse process of the seventh cervical vertebra and the neck of the first rib, dorsally from the

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subclavian artery (*a. subclavia*) and the beginning of the vertebral artery (*a. vertebralis*) [Sobociński 1985]. In man this ganglion originates from the fusion of the inferior cervical ganglion (*ganglion cervicale inferius*) with the first thoracic ganglion. It is situated between the transverse process of the seventh cervical vertebra and the neck of the first rib behind the subclavian artery [Collins 1991, Marciniak and Ziółkowski 1992]. The cervicothoracic ganglion in man is large, irregularly star-shaped and flat; laterally it is bounded by the thyrocervical trunk (*truncus thyrocervicalis*) and covered by the endothoracic fascia (*fascia endothoracica*) and parietal pleura (*pleura parietalis*) [Bochenek and Reicher, 2002]. The cervicothoracic ganglion in cat is 3–4 mm wide and stellate shape. It is situated into the left and right side in 1st intercostal space, under the longus colli muscle [König 1992]. Perman [1924] described the nerves supplying the heart in the pig, which branch off from the cervicothoracic ganglion. The study was a continuation of the research conducted by Pospieszny [1987], Klećkowska et al. [2003] and Klećkowska-Nawrot et al. [2007]. The subject of these studies was the morphological analysis of the ganglion in the pig from the 9th week of gestation [Klećkowska et al. 2003] and the 10th week of gestation [Pospieszny 1987] and in fetuses of the American Staffordshire terriers from the 62nd day of gestation [Klećkowska et al. 2007].

MATERIAL AND METHODS

The studies were conducted on 20 newborn Persian cats [7 males and 13 females] constituting the contents of 14 uteri. The material came from the 58th day of gestation. The infant cats were born dead or died soon after the delivery. Their age was determined on the basis of the CRL (crown-rump length) index [Beck et al. 1990]. The sections for examinations were kept in 4% solution of formic formaldehyde. The examinations were carried out using the method of macroscopic preparation with a forehead magnifying glass and binocular (magnification 1.5–5.0x). In order to better visualize the cervicothoracic ganglia and other accompanying anatomical structures 0.5–2% acetic acid solution was used for the examinations. The measurements of the ganglion were performed with the aid of an electronic caliper to an accuracy of 0.01 mm. The methods used in the topographic anatomy holotomy and skeletotomy were employed in the study. The Nomina Anatomica Veterinaria [2005] was used in the descriptive part. Photographic and tabular documentation was made.

RESULTS

Holotomy. The cervicothoracic ganglion in Persian cats coming from the 58th day of gestation is situated in C₇₋₈–Th₁₋₃ segment. It is formed from the fusion of three or four ganglia: the caudal cervical ganglion (Gcca), first thoracic ganglion (GTh₁), second thoracic ganglion (GTh₂), third thoracic ganglion (GTh₃). In four females (in 2 – on the left side, in 1 – on the right side and in 1 – on both sides) the cervicothoracic ganglion is formed from the fusion of the caudal cervical ganglion with the first and second thoracic ganglia. The cervicothoracic ganglion has a communicating ramus with the middle cervical ganglion (*ganglion cervicale medium*). The cervicothoracic ganglion lies laterally,

under the longus colli muscle (*m. longus colli*), over the trachea (*trachea*); it is covered by the endothoracic fascia and parietal pleura.

Skeletotopy. The cervicothoracic ganglion was characterized by the variable location relative to the cervical and thoracic segments of the vertebral column: - at the level C_7 - Th_1 to the cranial margin of the 2nd rib (1 case), - at the level of the 1st rib (3 cases), - at the level of the 2nd rib (1 case), - at the level from the 1st to the 2nd rib (11 cases), - at the level from the 1st to the 3rd rib (1 case), - between the cranial margins of the 1st and 2nd rib (4 cases), - between the cranial margins of the 1st and 3rd rib (2 cases), - at the level of the caudal margin of the 1st rib and the cranial margin of the 2nd rib (12 cases), - between the cranial margins of the 1st and the caudal margin of the 3rd rib (5 cases) (Fig. 1). As regards the relationship between the location of the cervicothoracic ganglion and the body side it was found that most frequently it was situated at the level of the caudal margin of the 1st rib and the cranial margin of the 2nd rib on the left side, while on the right side in 8 cases it was situated between the cranial margin of the 1st rib and the caudal margin of the 2nd rib. But as regards the location of the ganglion in relation to the sex of the examined animals it was found that in females it was most frequently (6 cases) situated at the level of the caudal margin of the 1st rib and the cranial margin of the 2nd rib, while in males it was also situated in 6 cases at the level of the caudal margin of the 1st rib and the cranial margin of the 2nd rib and between the cranial margin of the 1st rib and the caudal margin of the 2nd rib.

Shape. The shape of the examined ganglion was also determined. The cervicothoracic ganglion displayed one of three kinds of shape: oval (16 cases), oblong (12 cases), triangular (11 cases) and in one case it was oblong with a distinct narrowing (GCca+GTh₁ + GTh₂₋₃). On the left side of the body it was the oval ganglion that was most frequently found (10 cases). On the right side the oblong ganglion was the most frequent (8 cases). No distinct relationship between the ganglion shape and the sex was found. In females the oval shape occurred in 10 cases, oblong and triangular - in 8 cases. In males the oval shape was found in 6 cases, oblong - in 4 cases, triangular - in 3 cases, oblong with a narrowing - in 1 case.

Morphometry. The dimensions (length and width) considering the sex and the body side are presented in Tables 1 and 2. The morphometry of the examined cervicothoracic ganglion showed that the longest cervicothoracic ganglion is found on the left side in females ($\bar{x} = 3.016$ mm), the shortest on the right side also in females ($\bar{x} = 2.790$ mm). The widest ganglion occurs in males on the right side ($\bar{x} = 1.758$ mm), while the narrowest ganglion is found in females on the left side ($\bar{x} = 1.418$ mm).

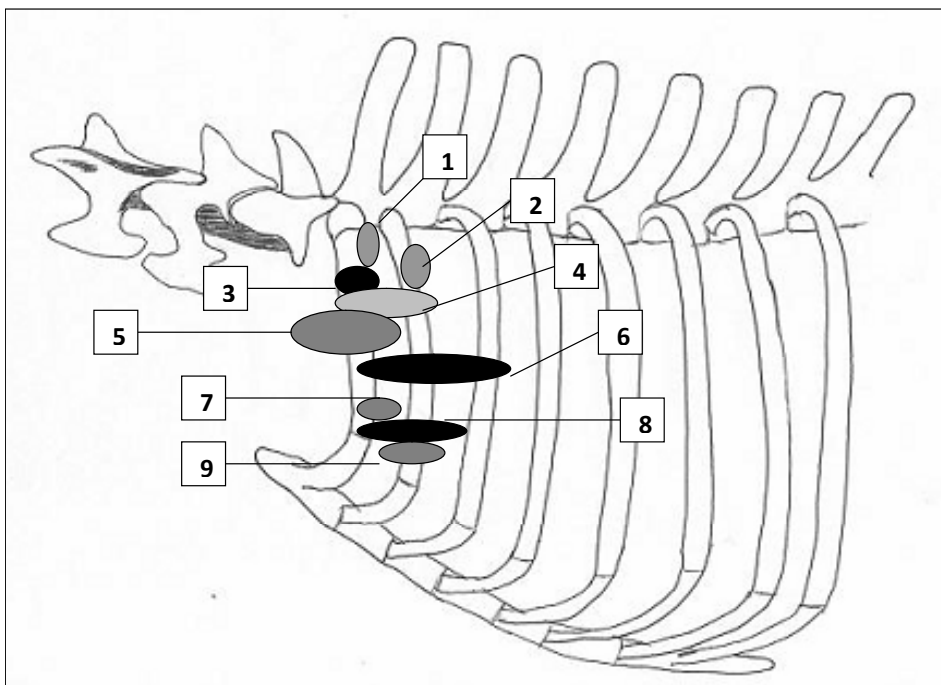


Fig. 1. The skeletotopy of cervicothoracic ganglion in newborn Persian cats

The cervicothoracic ganglion was characterized by the variable location relative to the cervical and thoracic segments of the vertebral column on the left and right side (left + right side = total)

1 – at the level of the caudal margin of the 1st rib and the cranial margin of the 2nd rib (12 cases), **2** – at the level of the 2nd rib (1 case), **3** – at the level of the 1st rib (3 cases), **4** – at the level from the 1st to the 2nd rib (11 cases), **5** – at the level C₇ to the cranial margin of the 2nd rib (1 case), **6** – at the level from the 1st to the 3rd rib (1 case), **7** – between the cranial margins of the 1st rib and the 2nd rib (4 cases), **8** – between the cranial margins of the 1st rib and 3rd rib (2 cases), **9** – between the cranial margin of the 1st and the caudal margin of the 3rd rib (5 cases)

Ryc. 1. Skeletotopia zwoju szyjno-piersiowego u noworodków kotów rasy pers.

Zwój szyjno-piersiowy charakteryzował się różnym położeniem w stosunku do odcinków szyjnych i piersiowych kręgosłupa po lewej i prawej stronie ciała (strona lewa + strona prawa = łącznie).

1 – na wysokości od brzegu doogonowego 1 żebra do brzegu doczaszkowego 2 żebra (12 przypadków), **2** – na wysokości 2 żebra (1 przypadek), **3** – na wysokości 1 żebra (3 przypadki), **4** – na wysokości od 1 do 2 żebra (11 przypadków), **5** – na wysokości od kręgu szyjnego 7 do brzegu doczaszkowego 2 żebra (1 przypadek), **6** – na wysokości od 1 do 3 żebra (1 przypadek), **7** – pomiędzy brzegiem doczaszkowym 1 a 2 żebra (4 przypadki), **8** – pomiędzy brzegiem doczaszkowym 1 a 3 żebra (2 przypadki), **9** – pomiędzy brzegiem doczaszkowym 1 żebra a doogonowym brzegiem 3 żebra (5 przypadków)

Table 1. The length [mm] of the cervicothoracic ganglia in newborn Persian cats with respect side and sex ($\bar{x} \pm SD$)Tabela 1. Długość (mm) zwoju szyjno-piersiowego u noworodków kotów rasy pers z uwzględnieniem strony ciała i płci ($\bar{x} \pm SD$)

	Male (7) Samiec		Female (13) Samica	
	Left side Strona lewa	Right side Strona prawa	Left side Strona lewa	Right side Strona prawa
	2.81	3.86	3.45	2.78
	2.04	2.09	1.55	4.20
	2.70	4.44	2.50	2.40
	4.13	2.62	4.78	3.09
	3.40	3.10	2.57	1.95
	3.84	2.39	5.35	3.04
	1.91	2.18	2.05	2.57
			2.03	3.16
			2.36	1.92
			3.18	1.72
			2.80	2.46
			2.79	4.20
			3.81	2.31
\bar{x}	2.98	2.95	3.02	2.75
SD	0.583	0.869	1.097	0.808

Table 2. The width [mm] of the cervicothoracic ganglia in newborn Persian cats with respect side and sex ($\bar{x} \pm SD$)Tabela 2. Szerokość (mm) zwoju szyjno-piersiowego u noworodków kotów rasy pers z uwzględnieniem strony ciała i płci ($\bar{x} \pm SD$)

	Male (7) Samiec		Female (13) Samica	
	Left side Strona lewa	Right side Strona prawa	Left side Strona lewa	Right side Strona prawa
	1.53	2.16	1.48	1.11
	1.08	1.78	1.52	1.45
	1.40	1.22	1.36	1.48
	1.53	1.64	1.18	1.47
	1.20	1.55	1.58	1.72
	1.70	1.83	0.69	1.16
	1.61	2.13	1.40	1.80
			1.28	1.70
			1.37	1.81
			1.40	1.42
			1.48	2.06
			1.25	1.50
			1.92	2.60
\bar{x}	1.44	1.76	1.38	1.64
SD	0.224	0.329	0.192	0.274

Ganglion nerve connections. The cervicothoracic ganglion gives off the following nerve branches (Fig. 2, 3): the subclavian ansa (cranial and caudal branch) – it may branch off from $GC_{ca}+GTh_1+GTh_2$, $GC_{ca}+GTh_1+GTh_2+GTh_3$ ganglia; the vertebral nerve – it runs through the canal of the transverse processes of the cervical vertebrae and gives off communicating rami to the 2nd–7th cervical nerves; communicating rami to cervical and thoracic spinal nerves (*rami communicantes cum nervi spinales cervicales et thoracis*) – these are 1–4 small branches which communicate with C_{7-8} and Th_{1-8} ; communicating rami to the vagus nerve (*rami communicantes cum nervi vagi*) – they branch off from the cervicothoracic ganglion (in the dog – from the caudal cervical ganglion); their number is 1–5; communicating rami to the phrenic nerve (*rami communicantes cum nervi phrenici*), their number is 1–2; vascular branches: subclavian and vertebral and carotic communis arteries; visceral branches: thoracic cardiac and cervical cardiac nerves (*nervi cardiaci thoracici et cardiaci cervicales*) – they branch off from the middle cervical ganglion as 1–2 tiny twigs and from the thoracic and cervicothoracic ganglia; their number is 1–3; tracheal branches (*rami tracheales*) – they branch off from the cervicothoracic ganglion; their number is 1–3; esophageal branches (*rami esophagei*) – they branch off from the cervicothoracic ganglion; their number is 1–2.

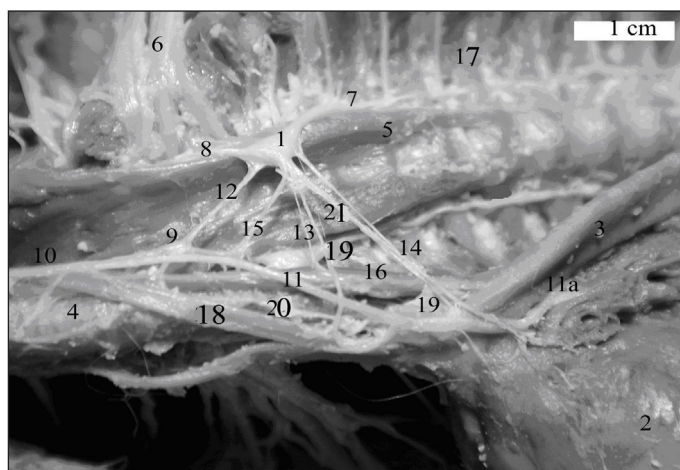


Fig. 2. The cervicothoracic ganglion in newborn Persian cats on the left side
 1 – cervicothoracic ganglion, 2 – heart, 3 – thoracic aorta, 4 – trachea, 5 – longus colli muscle, 6 – brachial plexus, 7 – sympathetic trunk, 8 – vertebral nerve, 9 – middle cervical ganglion, 10 – vagosympathetic trunk, 11 – vagus nerve, 11a – dorsal vagus nerve left, 12 – cranial branch of the subclavian ansa, 13 – esophageal branches, 14 – thoracic cardiac and cervical cardiac nerves, 15 – communicating rami to the vagus nerve, 16 – esophagus, 17 – 5th rib, 18 – left carotic communis artery, 19 – vascular branches, 20 – left recurrent laryngeal nerve, 21 – cardiac branches

Ryc. 2. Zwój szyjno-piersiowy u noworodków kotów rasy pers po stronie lewej ciała.
 1 – zwój szyjno-piersiowy, 2 – serce, 3 – aorta piersiowa, 4 – tchawica, 5 – mięsień długi szyi, 6 – splot ramienny, 7 – pień współczulny, 8 – nerw kręgowy, 9 – zwój szyjny środkowy, 10 – pień błędno-współczulny, 11 – nerw błędny, 11a – nerw błędny grzbietowy lewy, 12 – gałąź doczaszkowa pętli podobojczykowej, 13 – gałęzie przełykowe, 14 – nerwy sercowe piersiowy i szyjny, 15 – gałąź łącząca do nerwu błędnego, 16 – przełyk, 17 – 5 żebro, 18 – tętnica szyjna wspólna lewa, 19 – gałęzie naczyniowe, 20 – nerw krtaniowy powrotny lewy, 21 – gałęzie sercowe

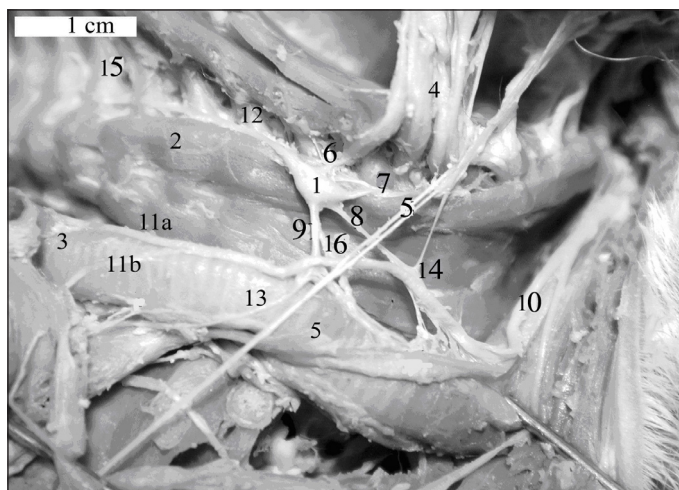


Fig. 3. The cervicothoracic ganglion in newborn Persian cats on the right side
 1 – cervicothoracic ganglion, 2 – longus colli muscle, 3 – trachea, 4 – brachial plexus, 5 – phrenic nerve right, 6 – vertebral nerve, 7 – communicating rami to cervical and thoracic spinal nerves, 8 – cranial branch of the subclavian ansa, 9 – caudal branch of the subclavian ansa, 10 – vagosympathetic trunk, 11a – dorsal branch vagus nerve, 11b – ventral branch vagus nerve, 12 – sympathetic trunk, 13 – thoracic cardiac nerves, 14 – middle cervical ganglion, 15 – 5th rib, 16 – subclavian ansa

Ryc. 3. Zwój szyjno-piersiowy u noworodków kotów rasy pers po stronie prawej ciała.

1 – zwój szyjno-piersiowy, 2 – mięsień długi szyi, 3 – tchawica, 4 – spleć ramienny, 5 – nerw przeponowy prawy, 6 – nerw kręgowy, 7 – gałęzie łączące do nerwów rdzeniowych piersiowych, 8 – gałąź doczaszkowa pętli podobojczykowej, 9 – gałąź doogonowa pętli podobojczykowej, 10 – pień błędno-współczulny, 11 – nerw błędny grzbietowy lewy, 12 – pień współczulny, 13 – nerwy sercowe piersiowe, 14 – zwój szyjny środkowy, 15 – 5^{ty} żebro, 16 – pętla podobojczykowa

DISCUSSION

Before starting the research on the cervicothoracic ganglion in fetuses of Persian cats it was assumed that its morphology and topography were similar to the morphology and topography of the cervicothoracic ganglion in the postnatal period in cats generally. Our research confirmed this thesis, although distinct differences were observed within one breed in the ganglion shape, its situation relative to the cervical and thoracic segment of the vertebral column, the kind of connections between the caudal cervical ganglion and the first, second and third thoracic ganglia, and in the ganglion morphometry, considering sex and the body side. According to Nickel et al. [2004] the cervicothoracic ganglion is formed in the cat from the fusion of the caudal cervical ganglion with the 1st–4th thoracic ganglion. It is situated at the level of the 1st intercostal space, laterally to the longus colli muscle. On account of the arrangement of its branches it is called the stellate ganglion. In the dog the cervicothoracic ganglion is the largest of all the sympathetic ganglia [Mizeres 1955]. It is situated on the dorsal part of the longus colli muscle extending between the 1st and 3rd rib. According to Kuder [2002] the cervicothoracic ganglion, in animals and

in man, is formed from the fusion of the caudal cervical ganglion and the first or second thoracic ganglion. It is a large ganglion situated between the transverse process of the 7th vertebra and the neck of the 1st rib. The nerves branching off from the cervicothoracic ganglion are: vertebral nerves which are formed from several nerve branches creating the vertebral plexus on the vertebral artery; vascular branches to the subclavian artery where they form the subclavian plexus; and visceral branches in the form of the inferior (caudal) cardiac cervical nerve (*n. cardiacus cervicalis inferior s. caudalis*) which begins with one or several roots and often combines with the medium cervical nerve (*n. cervicalis medius*) on the way to the heart. The cervicothoracic ganglion in cats in the postnatal period is 3–4 mm wide and displays the shape of an irregular star. It builds the subclavian ansa with the middle cervical ganglion [König 1992, Nickel et al. 2004]. This ganglion gives off the following branches: communicating rami to the 7th– 8th cervical nerve; communicating rami to the 3rd or 4th thoracic nerves; the vertebral nerve – as a root for the vertebral plexus; this nerve also forms a trunk for grey communicating rami to the 6th or 7th cervical nerves. The vertebral nerve is closely connected with the muscular branch for the longus colli muscle. This nerve arises from the cranial region of the cervicothoracic ganglion as a separate nerve or it unites with a ramus to the 7th cervical nerve and follows the medial surface of the vertebral artery into the canal of the transverse processes of the cervical vertebrae where it joins the vertebral plexus. The subclavian ansa gives off thoracic cardiac branches, which may separate from the subclavian ansa in the vicinity of the cervical cardiac nerves, next communicating rami to the recurrent laryngeal nerve and several branches to the subclavian plexus formed around the subclavian artery [Frewein and Vollmerhaus 1994]. According to Phillips et al. [1986] the right cervicothoracic ganglion in cats is relatively large and round. It is situated laterally to the longus colli muscle at the level of the 1st pair of ribs. It receives the branches from the lower cervical nerve and two upper thoracic nerves. The left cervicothoracic ganglion is situated in the dorsally lateral part of the mediastinum, laterally to the longus colli muscle in the vicinity of the 1st and 2nd rib. It is round and has communicating rami with the 1st and 2nd thoracic nerves and lower cervical nerves. The left and right vertebral nerve and the right dorsal and ventral subclavian ansa originate from the cranial part of the ganglion. The left dorsal subclavian ansa originates from the medial surface of the cranial part of the cervicothoracic ganglion. According to McKibben and Getty [1968] the cervicothoracic ganglion in domestic animals is formed from the fusion of the caudal cervical ganglion and two or three thoracic ganglia. It is situated on the right side laterally to the long cervical muscle at the level of the 1st intercostal space. On the left side it extends between the 1st and 2nd intercostal space. In the research conducted on five dogs, Dietz [1955] proved variability in situated of cervicothoracic ganglion. In two cases is found under 1st rib, and in three cases is situated under 3rd rib. In the research conducted on five dogs [Mizeres 1955] found that in four dogs on the left side the stellate ganglion gave off cardiac nerves while in one dog they originated from the ventral subclavian ansa. The subclavian ansa is formed by the splitting of the sympathetic trunk by the subclavian artery between the caudal cervical and stellate ganglia. The right subclavian ansa (*ansa subclavia dextra*) differs from the left subclavian ansa (*ansa subclavia sinistra*) because its ventral link may connect directly to the stellate ganglion or it may run within the sheath of the vagus nerve. The stellate ganglion lies on the dorsolateral aspect of the longus colli muscle extending between 1st and 3rd ribs. The above study has a cognitive and comparative character. It aimed at exa-

mining the analogy of the discussed parameters of the cervicothoracic ganglion in fetuses of Persian cats with the accessible results of the research conducted on adult individuals.

CONCLUSIONS

The research has shown that:

1. The cervicothoracic ganglion is formed from the fusion of three or four ganglia: the caudal cervical ganglion (GCca), first thoracic ganglion (GTh₁), second thoracic ganglion (GTh₂), third thoracic ganglion (GTh₃). It is situated in C₇₋₈-Th₁₋₃ segment.

2. It is characterized by variable location relative to the cervical and thoracic segment of the vertebral column (skeletotopy) and various shape (oval, oblong, triangular, oblong with a distinct narrowing).

3. The morphometry of the examined cervicothoracic ganglion proved that the longest cervicothoracic ganglion is found on the left side in females (\bar{x} = 3.016 mm), the shortest on the right side also in females (\bar{x} = 2.790 mm). The widest ganglion is found in males on the right side (\bar{x} = 1.758 mm), the narrowest in females on the left side (\bar{x} = 1.418 mm).

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BADANIA MAKRO ANATOMICZNE ZWOJU SZYJNO-PIERSIOWEGO U NOWORODKÓW KOTÓW RASY PERS

Streszczenie. Zwój szyjno-piersiowy lub gwiaździsty należy do części szyjnej układu współczulnego. U zwierząt powstaje on z połączenia zwoju szyjnego doogonowego ze zwojem piersiowym pierwszym, a często także ze zwojem piersiowym drugim. Badania przeprowadzono na 20 kociętach rasy pers [7 płci męskiej i 13 płci żeńskiej], pochodzących z 58. dnia ciąży. W wyniku przeprowadzonych badań stwierdzono, że zwój szyjno-piersiowy powstaje w następstwie zwartego połączenia się trzech lub czterech zwojów: szyjnego doogonowego (GCca), piersiowego pierwszego (GTh₁), piersiowego drugiego (GTh₂), piersiowego trzeciego (GTh₃). Położony jest na odcinku C_{7,8}–Th_{1,3}. Cechuje się on zmiennym położeniem w stosunku do odcinków szyjnego i piersiowego kręgosłupa (skeletotopia) oraz zmiennym kształtem (owalny, podłużny, trójkątny, podłużny z wyraźnym przewężeniem). Morfometria badanego zwoju szyjno-piersiowego wykazała, że ze względu na stronę ciała najdłuższy zwój szyjno-piersiowy występuje po stronie lewej u samic (3,016 mm), najkrótszy po stronie prawej także u samic (2,790 mm). Najszerszy zwój występuje natomiast u samców po stronie prawej (1,758 mm), najwęższy zaś u samic po stronie lewej (1,418 mm).

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INVASION OF *SETARIA TUNDRA* IN ROEDEER (*CAPREOLUS CAPREOLUS*) – CASE REPORT

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Abstract. *S. tundra* is a parasite of roe deer (*Capreolus capreolus*), reindeer (*Rangifer tarandus*) and moose (*Alces alces*). This parasite belongs to the genus *Setaria*, which includes more than 40 species, that are localised in the abdominal cavities of ruminants and equines. All these species produce microfilariae, which are present in host blood circulation. Our case report is the invasion of *Setaria tundra* in roe deer (*Capreolus capreolus*) in Lower Silesia. The 4 years old female of roe deer was found dead in forest in February 2010. There were found 12 females and 2 males in abdominal cavity. The histological examination of tissue slabs from lung showed presence of microfilaria in the lumen of vessels as well as blood smears.

Key words: filaria, *Setaria tundra*, roe deer (*Capreolus capreolus*)

INTRODUCTION

The *Setaria tundra* belongs to the genus *Setaria*, which includes more than 40 species, that are localized and parasitised in the abdominal cavities of ruminants and equines. All these species produce microfilariae, which are present in host blood circulation.

S. tundra is a parasite of roe deer (*Capreolus capreolus*), reindeer (*Rangifer tarandus*) and moose (*Alces alces*). Adult females are about 5,5–7,9 cm long and male are smaller, about 2,8–3,5 cm. Adult females are characterized by a peribuccal crown with two non-cuticularized lateral lips and a rounded mouth opening. The posterior end has terminal spikes and two very small, lateral, subterminal caudal appendages about 47 μm from the extremity. The male has several coils at the posterior extremity, whose ventral surface has a single pre-cloacal papilla and eight paired papillae. The spicules are about 65 and 600 μm in length [Liang-Sheng 1959, Shoho 1959].

An adult female of *S. tundra* produces thousands of larval stages (microfilariae) daily [Nelson 1966]. Microfilariae are taken up in the blood by the insect vectors. The vectors for *S. tundra* are the mosquitoes (*Anopheles*, *Aedes* and *Culex*) [Rehbinder 1990]. In the

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organism of the insect, parasite penetrate the gut wall and migrate to the haemocoel. Parasite develop into an infective third stage larva (L-3) in a certain tissue and then migrates along the haemocoel to the mouthparts of the vector [Anderson 2000, Bain and Babayan 2003].

There is little known about the life cycle of *Setaria* spp. and their migration to the abdominal cavity in the definitive host [Anderson 2000]. There is unknown the prepatent time for *S. tundra*, but in species belong to the genus *Setaria* this time is as long as one year or more: 224 days for *S. cervi*, approximately one year for *S. marshalli* [Fujii et al. 1996].

MATERIAL AND METHODS

The case involved roe-deer (*Capreolus capreolus*) from the Lower Silesia in Poland. The four years old female of roe deer was found death, in forest in February 2010. During that winter time there was very low average temperatures and deep snow cover (40 cm). There were made section of roe-deer. Samples of the organs (liver, spleen, kidney, lungs, intestines) taken to the histopathological and microbiology. The samples for histopathology were fixed in 10% formaldehyde solution, embedded in paraffin, cut into slabs and then HE (hematoxillin and eosin) staining were undertaken. Parasites from the peritoneal cavity were collected using dissecting pens, preserved in 70% ethanol. There were made, as well, cytological examination of blood smears from heart stained using Heamacolor (Merck). A standard set of organs (lung, liver, spleen, kidney, and small intestine) were examined for the presence of bacteria. Each sample was investigated by inoculating standard microbiological mediums (Columbia blood agar with 5% defibrinated sheep blood, McConkey/XLD agar, Sabouraud medium, brain-heart-infusion – Oxoid).

RESULTS

In roe deer a **strong emaciation** was observed. At necropsy, fourteen adult nematodes were collected in the peritoneal cavity. All the parasites found during this survey were morphologically identified as *Setaria tundra* (Fig. 1, 2). There were found 12 females and 2 males of 6,7–7,3 cm and 3,2–3,4 cm respectively. The histological examination of a samples taken from lung showed presence of microfilaria in the lumen of vessels (Fig. 3) as well as blood smears (Fig. 4). No pathogenic bacterial growth was found.



Fig. 1. The anterior end of female with two lateral lips and a rounded mouth opening (magnification 100x)

Ryc. 1. Przednia część ciała samicy z widocznymi dwoma bocznymi wargami i owalnym otworem gębowym (powiększenie 100x)



Fig. 2. The posterior end of female with two lateral, subterminal caudal appendages 47 μ m (magnification 100x)

Ryc. 2. Tylna część ciała samicy z widocznymi dwoma bocznymi wyrostkami o długości 47 μ m (powiększenie 100x)

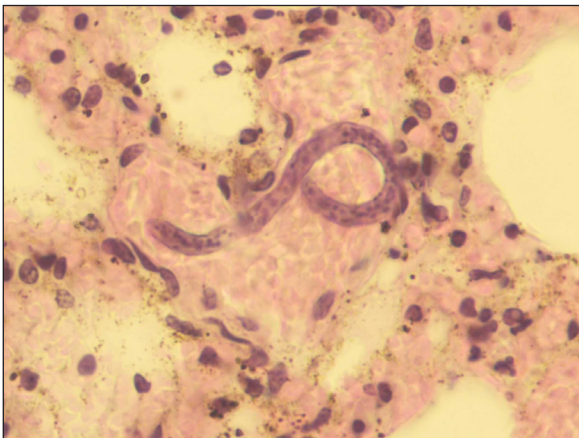


Fig. 3. The histopathological section of lung of roe-deer with microfilaria cells (H-E, magnification 200x)

Ryc. 3. Przekrój przez mikrofilarię w naczyniu żylnym sarny (barwienie H-E, powiększenie 200x)



Fig. 4. Microfilaria in smears (Hemacolor, magnification 200x)

Ryc. 4. Mikrofilarie, rozmaz (Hemacolor, powiększenie 200x)

DISCUSSION

Setaria tundra has been reported in roe deer from Germany, Austria, Italy and Poland [Boch and Schneidawind 1999, Buttner 1975, Drózdź 1966, Favia et al. 2003]. Invasion of this species was reported by Laaksonen et al. [2009] in reindeer.

We didn't observe negative effect of *S. tundra* in macroscopic examination (peritonitis) and in histopathology, but the harmful effects of *Setaria* on their hosts depend on number of parasites. Reindeer calves with heavy infections of *S. tundra* expressed weakness, poor body condition, and an undeveloped winter coat. In the macroscopic examinations, there is revealed peritonitis – ascites fluid, green fibrin deposits, adhesions, and presence of live and dead *S. tundra* nematodes. Histopathology indicated granulomatous peritonitis with lymphoplasmacytic and eosinophilic infiltration. Moreover, invasions could be associated with an outbreaks of peritonitis in wild cervids, for example in 1973 and 2003 in Finland. During outbreaks in 1973 tens of thousands of reindeer died in the northern part of this country. At this moment invasion of *S. tundra* cause serious economic problem in Finland [Laaksonen et al. 2009]. Invasion of *Setaria* sp. as an mosquito-borne diseases could be sensitive to weather and climate change. Future outbreaks in Poland can be predicted based on the mean temperatures.

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INWAZJA *SETARIA TUNDRA* U SARNY (*CAPREOLUS CAPREOLUS*) – OPIS PRZYPADKU

Streszczenie. *Setaria tundra* jest pasożytem saren (*Capreolus capreolus*), reniferów (*Rangifer tarandus*) i łośi (*Alces alces*). Do rodzaju *Setaria* zaliczanych jest ponad 40 gatunków, które pasożytują w jamie brzusznej przeżuwaczy i koniowatych. Wszystkie gatunki charakteryzuje produkcja mikrofilarii, które przedostają się do krwi żywicieli. Celem pracy jest przedstawienie przypadku inwazji filarii z rodzaju *Setaria* u sarny pochodzącej z Polski z terenu Dolnego Śląska. W trakcie badania sekcijnego martwej, czteroletniej sarny, która została znaleziona w lesie w lutym 2010 r., stwierdzono obecność w jamie brzusznej zwierzęcia 14 pasożytów (12 samic i 2 samców). Na podstawie cech morfologicznych pasożyt został sklasyfikowany do gatunku *Setaria tundra*. W wykonanych rozmazach krwi oraz przekrojach histopatologicznych naczyń krwionośnych płuc sarny widoczne były mikrofilarie.

Słowa kluczowe: filaria, *Setaria tundra*, sarna (*Capreolus capreolus*)

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