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AN IMPROVEMENT OF LAMB MEAT QUALITY BY SUPPLEMENTING WITH THERMALLY PROCESSED RAPE SEEDS (PART II)

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Abstract. The effect of supplementation with thermally processed rape seeds on the improvement of flavor and taste values of lamb meat was investigated in the present study. The study was conducted on rams of Polish Merino breed. The lambs from the control group received the CJ mixture and meadow hay and the lambs from the experimental group additionally 200 g/head/day of thermally processed rape seeds (heated for 30 minutes at the temperature of 120°C). After 90 days, all the animals were slaughtered and the samples of *musculus longissimus dorsi* were collected. Fatty acid profile was determined in intramuscular fat and sensory assessment of meat was also performed. It was found that the addition of thermally processed rape seeds resulted in a decrease in the content of saturated fatty acid (mainly stearic one C18:0) and an increase in the content of unsaturated fatty acids (mainly oleic acid C18:1) in intramuscular fat. The changes in the profile of fatty acids in intramuscular fat resulted in an improvement of flavor and taste values of lamb meat.

Key words: lamb, thermally processed rape seeds, fatty acids composition, sensory assessment

INTRODUCTION

At the current economic situation, the production of meat is the basic direction in the use of sheep in Poland. The research that has been carried out for many years on an improvement of this direction of utilization have been mainly focused on the intensification issue. However, there is scarcity of works, which aim would be an improvement of the quality of meat and an increase of its applicability as a consumption product.

In the case of sheep meat, the main factor that limits its larger demand is its specific flavor, evaluated by the majority of consumers as unpleasant. The results of the experiment demonstrated that the factor, which is the most responsible for this flavor, is the presence of significant quantity of higher saturated fatty acids, in particular of stearic ($C_{18:0}$) and palmitinic ($C_{16:0}$) acid and too low quantity of unsaturated fatty acids like oleic ($C_{18:1}$),

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linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acid [Herz and Chang 1970, Holfstrand and Jacobson 1960, Sink and Caparaso 1977]. However, many authors pointed out the possibility of an improvement of taste and flavor of lambs meat by nutrition means [Cramer et al. 1967, Field et al. 1983, Purchas et al. 1979].

The aim of the present work was to improve flavor and taste values of lamb meat by modification of fatty acids composition of intramuscular fat through the enrichment of feeding dose of lambs with thermally processed rape seeds (the treatment aimed at fat protection from lypolysis and unsaturated fatty acids protection from biohydrogenation in the rumen).

MATERIAL AND METHODS

Animal material consisted of 20 rams of Polish Merino breed. All the lambs were fed with the basic mixtures used in sheep diet, that is CJ mixture and meadow hay that were dosed according to the standards for fattening lambs. Additionally, lambs from the experimental group received the supplement of thermally processed rape seeds in the quantity of 200 g/head/day (caked seeds were heated at temperature of 120°C for 30 minutes) for the period of 90 days.

After 3 months, all the lambs were slaughtered and the samples of the *musculus longissimus dorsi* were collected. Intramuscular fat was extracted from the muscular tissue with Folch method (chloroform + methanol in a ratio 2:1). Fatty acids content was determined using capillary gas chromatography method on PU 4410 apparatus (Philips) with flame-ionization detector (FID) and 105 m long capillary column Rtx-2330 at Industrial Chemistry Research Institute, Warsaw, Poland.

Conditions of separation: initial isotherm – 160°C (30 min) – 3°C/min to 180°C – 17 min in temperature of 180°C, for 5 min to 210°C – 20 min in temperature of 210°C. Other conditions: temperature of column – 160°C, temperature of detector– 230°C, temperature of a chamber – 220°C, carrier gas – Helium 80 PSI.

Qualitative identification was conducted by the comparison of retention times of obtained peaks with retention times of Sigma-Aldrich Company standards.

Sensory assessment of muscular tissue was carried out at Department of Animal Products Technology and Quality Management, Wrocław University of Environmental and Life Sciences. Individual samples, wrapped in aluminum foil were baked in the dryer at the temperature 170°C until the temperature 72°C was reached in the geometrical center. The assessment was carried out by the team consisting of four persons according to the rules given by Tilgner [Baryłko-Pikielna 1975]. In the case of sensory assessment of color, taste and flavor, the criterion of intensity and desirability of a given attribute was applied. In the general assessment, only desirability was taken into consideration.

To assess the significance of differences between analysed parameters SAS (1996) statistical software was used.

RESULTS

The thermal processing of rape seeds did not influence on the content of fat and the profile of its fatty acids. The contribution of raw fat was on the average 45.6% and the

content of unsaturated fatty acids was about 88% (monounsaturated – 63.5%, polyunsaturated – 24.5%), and saturated ones about 12% (Tab. 1).

Table 1. The composition of rape seeds after thermally processed [%]
Tabela 1. Skład nasion rzepaku poddanych obróbce termicznej

Specification Wyszczególnienie	Content [%] Zawartość
Dry matter – Sucha masa	93.60
Protein – Białko	18.90
Raw fat – Tłuszcz surowy	45.65
Fatty acids – Kwasy tłuszczowe	
$C_{8:0}$	0.10
$C_{10:0}$	0.10
$C_{12:0}$	0.20
$C_{14:0}$	1.10
$C_{16:0}$	6.00
$C_{16:1}$	0.30
$C_{17:0}$	0.30
$C_{18:0}$	2.70
$C_{18:1}$	60.00
$C_{18:2}$	17.90
$C_{18:3}$	6.70
$C_{20:0}$	0.60
$C_{20:1}$	2.70
$C_{22:0}$	0.60
$C_{22:1}$	0.70

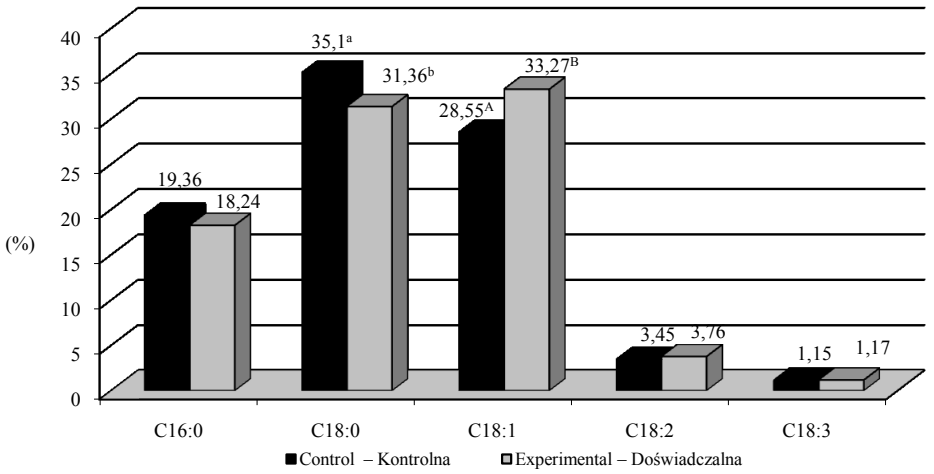
However, the result of lambs diet enrichment with the addition of thermally processed rape seeds was the increase in the total content of intramuscular unsaturated fatty acids in fat of 12.1% ($P \leq 0.05$) (monounsaturated acids of 13.1% ($P \leq 0.01$) and polyunsaturated acids of 5.4%), and a decrease in saturated acids content of 7% (Tab. 2).

Table 2. The composition of fatty acids of intramuscular fat of lambs from control group and group receiving the supplementation of thermally processed rape seeds (experimental) [%]
Tabela 2. Skład kwasów tłuszczowych tłuszczu wewnątrzmięśniowego jagniąt z grupy kontrolnej i z grupy otrzymującej dodatek nasion rzepaku poddanych obróbce termicznej (doświadczalna)

Fatty acids Kwasy tłuszczowe	Statistical symbol Parametry statystyczne	Groups – Grupy	
		control kontrolna	experimental doświadczalna
saturated – nasycone	**X** ± SD	**63.29** ± 1.96	**58.85** ± 1.66
unsaturated – nienasycone including – w tym	**X** ± SD	**36.71**^{a} ± 0.97	**41.15**^{b} ± 1.13
monounsaturated jednonienasycone	**X** ± SD	**31.89**^{A} ± 1.17	**36.07**^{B} ± 1.97
polyunsaturated wielonienasycone	**X** ± SD	**4.82** ± 0.26	**5.08** ± 0.32

a, b; A, B – differences significant on the level of $P \leq 0.05$ and $P \leq 0.01$
a, b; A, B – różnice istotne na poziomie $P \leq 0,05$ i $P \leq 0,01$

The application of thermally processed rape seeds also resulted in a decrease of the contribution of palmitinic ($C_{16:0}$) and stearic ($C_{18:0}$) acid in intramuscular fat, of 5.8% and 10.6% (P≤0.05), respectively, and an increase of the content of oleic acid ($C_{18:1}$) of 16.3% (P≤0.01), linoleic ($C_{18:2}$) of 8.9% and linolenic ($C_{18:3}$) of 1,7% (Fig. 1).



a, b; A, B – differences significant on the level of P≤0.05 and P≤0.01
a, b; A, B – różnice istotne na poziomie P≤0,05 i P≤0,01

Fig. 1. The content of fatty acids of intramuscular fat of lambs from control group and group receiving the supplemention of thermally processed rape seeds (experimental) [%]
Ryc. 1. Zawartość kwasów tłuszczowych w tłuszczu wewnątrzmięśniowym jagniąt z grupy kontrolnej i grupy otrzymującej dodatek nasion rzepaku poddanych obróbce termicznej (doświadczalna)

The result of the profitable changes in the profile of fatty acids, which were effect of thermally processed rape seeds feeding, was the improvement of taste and flavor characteristics of lamb meat (Tab. 3).

The meat of lambs which diet was supplemented with protected rape seeds was characterized with less intense flavor and improved taste, tenderness and juiciness. Also, general assessment of meat of lambs fed on the diet with thermally processed rape seeds was significantly more advantageous in comparison with lambs that did not receive this additive.

DISCUSSION

Organoleptic assessment is the final test of consumers' requirements satisfaction. The majority of consumers, assess taste and flavor of lamb meat as disadvantageous, unpleasant and undesired.

The fat is the main factor which determines the flavour of the products [Ford and Park 1980, Herz and Chang 1970, Sink 1973, 1979]. It is even thought that fat determines the taste of meat, since the flavor is the main component of taste [Forss 1969, Horstein and Crowe 1963].

Park and Thomas [1973] found that specific flavor of lamb fat is determined first of all by high content of saturated fatty acids, in particular palmitinic $C_{16:0}$ and stearic acid $C_{18:0}$. Ford et al. [1975] showed that in the case of lamb meat containing higher levels of linoleic acid, the increase of which was the result of feeding with formaldehyde protected sunflower seeds, was characterized by significantly different taste and flavor than the meat of lambs that did not receive this additive. Park et al. [1978] observed that the addition of protected seeds of oil plants may advantageously modify taste and flavor of mutton, giving it sweet and fruity taste. According to the data obtained by the same authors, changed taste and flavor of mutton was observed as the result of feeding with protected plant fats that was associated with an increase of the content of carbonyl compounds that are released during thermal processing of meat with elevated content of linoleic acid. However, in other studies, Park and Thomas [1973] found that through the increase of the content of unsaturated fatty acids and decrease of saturated fatty acids in sheep fat, meat and fat lost its disadvantageous flavor and taste and became similar to pork rather than to mutton. Similar observations were also performed by Park et al. [1975] who, as the result of lambs diet supplementation with protected sunflower seeds, obtained meat with significantly less intense flavor and taste. These changes resulted in the increase of the acceptance of this meat by these consumers who did not tolerate it previously.

In the present study, the diet of lambs was enriched with thermally processed rape seeds. The application of high temperature resulted in denaturation of protein seeds that resulted in a decrease of its susceptibility to proteolysis and deamination in the rumen. Thanks to that, a part of unsaturated fatty acids in the unchanged form passed through the rumen into the stomach. In the stomach, more acidic environment resulted in hydrolysis of protein shield and release of unsaturated fatty acids that were absorbed in small intestine.

As a result of the application of thermally protected rape seeds in lambs diet, the contribution of saturated fatty acids in intramuscular fat decreased (mainly of stearic acid $C_{18:0}$) and those of unsaturated ones (mainly oleic acid $C_{18:1}$) increased. These changes had the advantageous influence on flavor value of fat and taste value of meat. The meat of lambs fed with the thermally processed rape seeds supplement obtained higher tasting degree in the area of flavor, juiciness, tenderness and taste in comparison with the meat of lambs that were not fed with this supplement.

The advantageous effect of the application of protected fats of oil plants in order to improve the flavor of sheep fat, was also indicated by the results of another authors [Anne et al. 1975, Park et al. 1975, 1976].

CONCLUSIONS

1. The addition of thermally processed rape seeds caused the advantageous modification of the composition of fatty acid in intramuscular fat (the content of saturated fatty acids decreased and unsaturated acids increased).

2. The decrease of the contribution of palmitinic $C_{16:0}$ and stearic $C_{18:0}$ acids and increase in the content of oleic $C_{18:1}$ acid were associated with the improvement of flavor and taste characteristics of meat.

3. On the basis of the results obtained it is necessary to state that the result of the application of thermally protected oil plats in lambs diet, enables to improve the sensory characteristics of meat.

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POPRAWA JAKOŚCI MIĘSA JAGNIĘCEGO POPRZEZ SUPLEMENTACJĘ DIETY JAGNIĄT NASIONAMI RZEPAKU PODDANYMI OBRÓBCE TERMICZNEJ (CZĘŚĆ II)

Streszczenie. W badaniach oceniono wpływ dodatku termicznie preparowanych nasion rzepaku na poprawę walorów zapachowych i smakowych mięsa jagnięcego. Badania przeprowadzono na 20 tryczkach rasy merynos. Jagnięta z grupy kontrolnej otrzymywały mieszankę CJ i siano łąkowe, natomiast z grupy doświadczalnej dodatkowo 200 g/szt/dzień preparowanych termicznie nasion rzepaku (ogrzewane przez 30 min w temp 120°C). Po 90 dniach wszystkie zwierzęta poddano ubojowi i pobrano od nich próbki mięśnia najdłuższego grzbietu, w których oznaczono profil kwasów tłuszczowych tłuszczu wewnątrzmięśniowego oraz przeprowadzono ocenę sensoryczną mięsa. Stwierdzono, że dodatek preparowanych termicznie nasion rzepaku spowodował w tłuszczu wewnątrzmięśniowym spadek zawartości kwasów tłuszczowych nasyconych (głównie kwasu stearynowego C18:0) oraz wzrost zawartości kwasów tłuszczowych nienasyconych (głównie kwasu oleinowego C 18:1). Wynikiem zmian w profilu kwasów tłuszczowych była poprawa walorów zapachowych i smakowych mięsa jagnięcego.

Słowa kluczowe: jagnięta, nasiona rzepaku poddane obróbce termicznej, skład kwasów tłuszczowych, ocena sensoryczna

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THE EFFECTS OF DIETARY SUPPLEMENTATION WITH CLA AND *CAMELINA SATIVA* SEEDS OIL ON PERFORMANCE OF BROILER CHICKENS

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Abstract. The objective of this study was to evaluate the effect of dietary conjugated linoleic acid (CLA) and false flax (*Camelina sativa)* seeds oil (FO), as a replacement for sunflower oil, on the performance of broiler chickens. Experiment was carried out on 96 chickens randomly divided into four groups – control: fed on the diet with sunflower oil (SO) and three experimental: fed on the diet with addition of CLA (2,86% starter, 4,32% grower) and fed on the diet with addition of CLA (1,43% starter, 2,16% grower) and SO or FO (1,43% starter, 2,16% grower). Daily weight gain, daily feed consumption, feed conversion ratio as well as final body weight were measured. No significant differences in growth performance were observed in the first period of experiment. In older birds dietary treatment with SO+CLA and FO+CLA daily weight gain, feed consumption and final body weight slightly increased. No significant differences in the values of analyzed parameters between SO, CLA and SO+CLA groups were observed. In conclusion, the results indicated that false flax oil and CLA can replace sunflower oil in chickens' feeding, and demonstrated the effectiveness of false flax oil on the growth performance of broiler chickens.

Key words: broiler, chicken, CLA, *Camelina sativa*, growth performance

INTRODUCTION

The essential polyunsaturated fatty acids (PUFA) from n-6 and n-3 classes are currently being studied to understand their effects on different organs and their functions. The n-6 family is derived from linoleic acid (LA, 18:2n-6) and the n-3 family is derived from α-linolenic (ALA, 18:3n-3). LA and ALA are essential fatty acids (FAs) and are the precursors of long-chain (> 20-carbon) n-6 and n-3 fatty acids such as arachidonic (AA, 20:4 n-6) and eicosapentaenoic acids (EPA, 20:5 n-3). The bioconversion LA and ALA into long-chain polyunsaturated fatty acids (LCPUFA) depends on factors such as the concentration of n-6 and n-3 and the ratio of n-6 to n-3 in the diet.

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Conjugated linoleic acid isomers (CLAs) are a group of positional and geometric (*cis* or *trans*) isomers of linoleic acid characterised by the presence of conjugated double bonds [Pariza et al. 2001, Belury 2002]. Biological activity has been attributed to 9*cis,11tran*-octadecadienoic acid and 10*trans,12cis*-octadecadienoic acid, and both isomers are considered to modulate lipid metabolism [Pariza et al. 2001]. The 9*cis,11trans* and 10*trans,12cis* isomers of CLA are routinely found in animal tissues [Chin et al. 2001]. Numerous biological effects of CLA have been described [Pariza et al. 2001, Azain 2003, Pariza 2004].

Recent research shows that it is possible to use false flax seeds (*Camelina sativa, CS*) and its by-products for animal nutrition [Flachowsky et al. 1997, Jaśkiewicz and Matyka 2003, Peiretti et al. 2007]. The utilization of camelina products in poultry diets has already been studied [Zubr 1993, Jaśkiewicz and Matyka 2003, Frame et al. 2007, Ryhänen et al. 2007]. Camelina oil (FO), extracted from false flax seeds, is known to have a high content of PUFA (above 50%), especially of n-3 fatty acids such as ALA amounting to about 36–42 % of total fatty acids [Flachowsky 1997], and a high content of linoleic acid (about 16–24%). The fatty acids profile of this oil appears to be very interesting and have important implications from a nutritional point of view.

Since during the last years *Camelina sativa* has attracted renewed attention as an alternative oilseed crop, the aim of the present study was to evaluate the performance of chicken diets that integrated *Camelina sativa* oil and CLA as a replacement for sunflower oil.

MATERIAL AND METHODS

Animals and housing

The experimental procedures used throughout this study were approved by The 2nd Local Animal Welfare Committee at the University of Life Sciences in Lublin, Poland (nr 89/2009).

A total of 96 broiler chickens Ross 308 both sex were utilized in the study. Upon receipt from hatchery 1-day old broilers were sexed and raised in floor-cages, each with six birds (three males and three females).

Experimental procedure

Chickens were randomly allocated to each of the four dietary treatments, 4 replicates of 6 birds each. Feed and water were available *ad libitum* through the whole experimental period. The experimental trial started on day 1 and lasted 35 days. Commercially-sourced pre-starter feed was provided to the 10th day of age to all birds. From the 11th to 21st day of life chickens were fed on control and experimental starter diets, and in the next period on grower diets. The composition of the starter and grower diets are presented in Table 1. The diets were formulated to be isocaloric and to meet the nutrient requirements of broilers [Smulikowska and Rutkowski 2005]. The following dietary treatments were applied: 1) control diet, SO (SO, sunflower oil) 2) CLA (CLA, purified conjugated linoleic), 3) SO+CLA and 4) FO+CLA (FO, false flax seeds oil). Dietary treatments are presented in Table 2. Daily weight gain (DWG), daily feed consumption, feed conversion ratio (FCR) as well as final body weight were measured.

Table 1. Composition and calculated nutrients content of the broiler diets
Tabela 1. Skład surowcowy i wyliczona zawartość składników pokarmowych i energii metabolicznej w mieszankach dla kurcząt brojlerów

Item Wyszczególnienie	10-–21 day 10.–21. dzień	22–35 day 22.–35. dzień
Ingredients – Składniki [%]		
Corn – Kukurydza	30.00	30.00
Wheat – Pszenica	35.85	35.26
Soybean meal – Śruta sojowa, 46%	24.50	22.45
Rapeseed meal – Śruta rzepakowa, 35%	3.00	5.00
Oil [1] – Olej	2.86	4.33
Limestone – Kreda	1.20	0.77
Calcium phosphate – Fosforan wapnia	0.95	0.89
Lysine – Lizyna	0.41	0.26
Methionine – Metionina	0.30	0.21
Threonine – Treonina	0.14	0.08
Sal – Wodorowęglan sodu	0.29	0.25
Mineral and vitamin premix[2] – Premiks mineralno-witaminowy	0.50	0.50
Total – Całość	100	100
Calculated ingredients – Składniki kalkulowane		
Protein crude – Białko ogólne [%]	18.70	17.61
ME – Energia metaboliczna [MJ/kg]	13,20	13,40
Ether extract – Ekstrakt eterowy [%]	6.50	7.00
Crude fiber – Włókno surowe [%]	2.99	3.15
Ash – Popiół [%]	4.75	4.63
Calcium – Wapń [g/kg]	9.00	8.50
Available Phosphorus – Fosfor przyswajalny [g/kg]	4.50	4.20

[1] SO, SO+CLA, CLA, FO+CLA (SO – sunflower oil, CLA – conjugated linoleic acid, FO – false flax oil)
[1] SO, SO+CLA, CLA, FO+CLA (SO – olej słonecznikowy, CLA – sprzężony kwas linolowy , FO – olej z lnianki)
[2] The premix supplied per kilogram of feed (labelec on the bag): 11 000 IU of vitamin A, 2 800 IU of vitamin D_3, 40 mg of vitamin E, 2.8 mg of vitamin K_3, 1,5 mg of vitamin B_1, 5 mg of vitamin B_2,35 mg of nicotin acid, 12 mg of pantothenic acid, 3 mg of vitamin B_6, 22 µg of vitamin B_{12}, 0.16 mg of biotin, 450 mg of choline, 1 mg of folic acid, 54 mg of Fe, 81 mg of Mn, 72 mg of Zn, 0.9 mg of I, 0.6 mg of Co, 30 mg of Se
[2] Dodatek premiksu na kilogram paszy (opis na opakowaniu): 11 000 IU witaminy A, 2 800 IU witaminy D_3, 40 mg witaminy E, 2,8 mg witaminy K_3, 1,5 mg witaminy B_1, 5 mg witaminy B_2, 35 mg kwasu nikotynowego, 12 mg kwasu pantotenowego, 3 mg witaminy B_6, 22 µg witaminy B_{12}, 0,16 mg biotyny, 450 mg choliny, 1 mg kwasu foliowego, 54 mg Fe, 81 mg Mn, 72 mg Zn, 0,9 mg I, 0,6 mg Co, 30 mg Se

Table 2. Experimental procedure (oil contents of diets, [%])
Tabela 2. Układ doświadczenia (zawartość oleju w paszy, [%])

Groups Grupy	Starter diet			Grower diet		
	SO	CLA	FO	SO	CLA	FO
SO	2.86	–	–	4.33	–	–
CLA	–	2.86	–	–	4.33	–
SO+CLA	1.43	1.43	–	2.165	2.165	–
CLA+FO	–	1.43	1.43	–	2.165	2.165

SO – sunflower oil, CLA – conjugated linoleic acid, FO – false flax oil
SO – olej słonecznikowy, CLA – sprzężony kwas linolowy, FO – olej z lnianki

Fatty acid analyses

The oils were sampled before mixing the control and experimental diets. Samples were saponificated with methanol solution of NaOH. The salts of fatty acids were esterified anhydrous methanol, using BF_3 as a catalyst and the methyl esters of fatty acids were analyzed on a gas chromatograph with a FID detector, with the use of a packed column (length 2.5 m, ID 4 mm, Gaz-Chrom packing, 80/100 mesh) according to the method described by Matyka [1976]. Fatty acids were identified by comparing their retention times with those of the corresponding standards (Sigma-Aldrich). CLA isomers content in mixture was declared by the producer – BASF The Chemical Company (Poland).

Statistical analysis

The results are presented as mean ±SE. Data were statistically analyzed by one-way analysis of variance (Anova) with the aid of Statistica 5.0 software. Differences among each treatment group were tested by Tukey's multiple comparison test, and were considered significant at $P \leq 0.05$.

RESULTS

False flax oil and sunflower oil were studied to determine the fatty acids composition (Tab. 3). The main FAs components in sunflower oil were linoleic acid (18:2n-6) – 62.23% and oleic acid (18:1n-9) – 25.30%, whereas in false flax oil they were α-linolenic acid (18:3n-3) – 36.08%, linoleic acid (18:2n-6) – 16.70% and eicosenoic acid (20:1n-11) – 14.56%.

Table 3. Fatty acid composition of oils used in the experiment [%]
Tabela 3. Skład kwasów tłuszczowych w olejach użytych w doświadczeniu

	SO	CLA	FO
C 16:0	6.01	–	5.06
C 18:0	3.57	–	2.69
C 18:1n-9	25.30	–	15.74
C 18:2n-6	62.23	–	16.70
c9, t11 min.	–	28	–
c10, t12 min.	–	28	–
assay CLA min.	–	56	–
C 18:3n-3	–	–	36.08
C 20:1n-11	0.31	–	14.56
C 20:3n-6	–	–	1.51
C 20:5n-3	–	–	1.51
SFA	10.58	–	9.90
MUFA	25.90	–	33.74
PUFA	62.23	–	55.80
PUFAn-6/n-3	–	–	0.47:1

The effect of feeding broiler chickens with SO, SO+CLA, CLA and FO+CLA is shown in Table 4. In both periods of experiment DWG was higher in SO+CLA and FO+CLA fed birds compared with the birds fed the diets of SO and CLA. However, these differences were not significant. There was no significant difference between daily feed consumption and FCR when birds were fed the control and experimental diet in the first period of experiment. Older chickens fed the diet with SO+CLA and FO+CLA had greater daily feed intake than those fed on diets with SO or CLA, however no significant differences in FCR were observed. Final body weight were higher in SO+CLA and FO+CLA than SO and CLA groups.

Table 4. Performance of broiler chickens (mean ±SE)

Tabela 4. Wskaźniki odchowu kurcząt brojlerów (średnia ±SE)

	SO	CLA	SO+CLA	FO+CLA
Age 11–21 day Wiek 11–21 dni				
Daily weight gain [g] Dzienny przyrost masy ciała	68.72±5.10	67.25±4.85	70.37±4.98	69.02±6.28
Daily feed consumption [g] Dzienne spożycie paszy	99.35±7.86	101.00±8.31	102.55±8.55	100.57±8.15
Feed conversion ratio [g/g] Zużycie paszy	1.44±0.03	1.50±0.02	1.46±0.06	1.46±0.03
Age 22–35 day Wiek 22–35 dni				
Daily weight gain [g] Dzienny przyrost masy ciała	81.01±6.68	78.23±6.41	82.22±6.07	82.55±7.12
Daily feed consumption [g] Dzienne spożycie paszy	143.69±10.09	142.50±19.06	150.33±14.12	149.43±16.03
Feed conversion ratio [g/g] Zużycie paszy	1.77±0.03	1.82±0.18	1.83±0.08	1.81±0.04
Final weight [kg] Końcowa masa ciała	2.115±0.31	2.035±0.30	2.159±0.22	2.150±0.25

DISCUSSION

The fatty acids profile of used sunflower oil and false flax oil was according to the expected and similar to the composition presented by other authors [Givens et al. 2001]. False flax oil is rich in essential FAs and is also qualitatively different from the more common vegetable oils with high PUFA proportions, such as soya oil, sunflower oil etc. Apart from *Camelina sativa,* ALA is found only in linseed and edible fish oils [Crowley 1998], but compared to linseed oil, camelina oil has a lower content of this acid but also a lower content of saturated fatty acids.

Current data indicate different influence of camelina oil and its by-products on animals growth. Ryhänen et al. [2007] observed that *Camelina sativa* expeller (5 or 10%) reduced the growth of chickens, depressed their feed intake and feed conversion ratio

during the starter phase. The young turkeys fed the diet in which camelina oil replaced vegetable oil showed comparable growth and feed conversion to the control group [Frame et al. 2007]. No statistically significant differences in final BW and feed conversion after camelina meal feeding were observed. However, the trend for BW to decrease with increasing dietary content of camelina meal was consistent. Also, no effect on the growth performance was observed in mammals. In studies of Peiretti et al. [2007] there were no significant differences in BW, weight gain, feed consumption and feed efficiency in rabbits after dietary treatment of various levels of *Camelina sativa* seeds. Moreover, results obtained by Rokka et al. [2002] show that the fatty acid composition of hen eggs can be beneficially modified by *Camelina sativa* oil. Feeding of *CS* oil increased the content of n-3 fatty acids, especially ALA. Feeding *C. sativa* modified the fatty acid composition in abdominal fat of broiler chickens, showing low ratio of n-6 to n-3 FA and a high level of ALA [Jaśkiewicz and Matyka 2003]. Similar changes in fatty acid composition in broiler meat as well as in pig meat can also be induced by feeding *CS* expeller [Flachowsky et al. 1997, Ryhänen et al. 2007].

The purpose of this study was to investigate the effects of CLA mixture and false flax oil in the diets of broiler chickens on growth performance. No significant differences in growth performance were observed in the first period of experiment. However, daily weight gain oscillated towards higher values in chickens fed on the SO+CLA and FO+CLA oils. In older birds dietary treatment with SO+CLA and FO+CLA slightly increased the DWG, feed consumption and final body weight. These data indicate that a mix of false flax oil and CLA can replace sunflower oil in chickens feeding, because no negative changes in growth and performance were observed.

The effects of dietary CLA supplementation on growth and performance of broiler chickens were investigated in recent years. In the study performed by Suksombat et al. [2007] daily weight gain was significantly decreased by dietary CLA, feed conversion ratio increased with increasing CLA (0.5–1.5%), whereas no effects on daily feed intake were observed. Badinga et al. [2003] reported that weight gain decreased with the concentration of CLA in the diet. In contrast with this paper, Bolukbasi [2006] demonstrated that broilers given 1, 2, or 3% CLA exhibited increased body weights and rates of body weight gain. Marked reduction of weight gain and feed conversion was reported by Szymczyk et al. [2001]. No significant differences in feed efficiency of the birds were observed by Du and Ahn [2002], Javadi et al. [2007] and Kawahara et al. [2009]. Kim et al. [2009] observed no significant alterations in growth performance, although average daily gain and average daily feed intake were generally higher in the chicken receiving 2% purified CLA. The results of Zhang et al. [2007] showed that CLA supplementation did not significantly influence body weight, but increased feed conversion.

In the present study, CLA treatment alone did not influence significantly the daily weight gain, daily feed consumption and FCR. However, depressed daily weight gain and worse FCR in both periods of experiment were observed. The final body weight of CLA broilers was lower by 3.8%, 5.7% and 5.3% than SO, SO+CLA and FO+CLA groups, respectively. The obtained results indicate that fattening mix feeds with SO+CLA or FO+CLA compositions may be more efficient than with CLA alone.

Therefore, feeding periods, dietary concentration, and type of isomer CLA may be the factors affecting growth performance. Bolukbasi [2006] reported that the percentage of arachidonic acid in tissues from hens fed on diets enriched with CLA was lower than from

hens fed on the diet without CLA, probably because of the competition of metabolites of dietary CLA isomers with metabolites of linoleic acid, particularly in reaction with D6-, D5-desaturases and elongase.

CONCLUSIONS

– There were no significant differences in basic production parameters between groups receiving different oils.
– Camelina sativa oil may be integrated alone or with CLA into broiler chickens diets without any adverse effect on growth performance of broiler chickens.

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WPŁYW DODATKU CLA I OLEJU Z NASION LNIANKI SIEWNEJ (*CAMELINA SATIVA*) NA PARAMETRY ODCHOWU KURCZĄT BROJLERÓW

Streszczenie. Celem pracy było zbadanie wpływu żywienia paszą zawierającą sprzężony kwas linolowy (CLA) i olej z lnianki siewnej (*Camelina sativa*) (FO) na wskaźniki odchowu kurcząt brojlerów. Doświadczenie przeprowadzono na 96 kurczętach podzielonych na cztery grupy – kontrolną, żywioną paszą z olejem słonecznikowym (SO) i trzy doświadczalne: grupa żywiona paszą z dodatkiem CLA (2,86% starter, 4,32% grower) lub paszą z dodatkiem CLA i SO (1,43% starter, 2,16% grower), bądź CLA i FO (1,43% starter, 2,16% grower). Oznaczono dzienne przyrosty, spożycie paszy, zużycie paszy oraz masę końcową. Podczas pierwszego okresu odchowu nie zaobserwowano znaczących różnic we wskaźnikach odchowu. U starszych ptaków żywienie mieszankami z dodatkiem SO+CLA i FO+CLA nieznacznie podniosło dzienne przyrosty, spożycie paszy i masę końcową. Nie stwierdzono istotnych różnic badanych parametrów między grupami SO, CLA i SO+CLA. Reasumując, uzyskane wskaźniki odchowu potwierdziły przydatność oleju z lnianki siewnej i CLA, stosowanych w miejsce oleju słonecznikowego, w żywieniu kurcząt brojlerów.

Słowa kluczowe: brojlery, kurczęta, CLA, Camelina sativa, wzrost

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USEFULNESS OF BLOOD FILMS FOR THE FELINE INFECTIOUS ANAEMIA DIAGNOSIS

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Abstract. Feline infectious anaemia (FIA) is a disease caused by a microbe called *Mycoplasma haemofelis. Mycoplasma haemofelis* is an extracellular parasite that lives on the surface of erythrocytes of domestic cats but is not limited to them (*Felis catus, Felis domestica).* In order to identify the parasite in blood, a blood smear test cat is carried out by means of Romanowski staining methods acridine orange staining (which stains bacterial DNA and RNA) and direct fluorescent antibody staining methods.

The aim of this study is to show that the performance of blood smearing in suspected animals is useful for the FIA diagnosis.

The result showed that it is impossible to make a definite diagnosis of FIA on the basis of clinical symptoms as it was found in only 80% of the cats with the symptoms of apathy, loss of appetite, cachexia, fetor ex ore, lymph nodes enlargement, mucosal ischaemia, mucosal icterus, arouse a much suspicion of the disease and may call for a further procedure. Performance of blood smearing seems to be a good diagnostic method in the case of the FIA disease, yet, it should be carried out at least three times at interval over several days.

Key words: Feline infectious anaemia (FIA), *Mycoplasma haemofelis*, cat, blood films

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INTRODUCTION

Feline infectious anaemia (FIA) is a disease caused by a microbe *Mycoplasma haemofelis* (the previous name – *Haemobartonella felis*) (Maede and Hata 1975, Maede 1975). Bacteria are spherical, bacillary and grain-like in shape and can form the chains of cells. The size of a bacillus is 0.2–0.5 µm in diameter, and 0.1–0.8 µm in lenght (Harvey 2001, Kurzeja 1979). These microbes has no cilia, flagella, nucleus or any other intracellular structures, nor does it have a rigid cell wall [Camey and England 1993, Carney and England 1993, Clark et al. 2002, Woyciechowska and Frygin 1978]. These microbes have a single or double cell membrane and they are Gram-negative [Kurzeja 1979]. They belong to the *Rickettsiaceae* family and *Rickettsia* genus. Other representatives of the family include *Anaplasma, Aeytianella, Paranaplasma,* and *Eperythrozoon.*

Mycoplasma haemofelis is an extracellular parasite that lives on the surface of erythrocytes of domestic cats (*Felis catus, Felis domestica)* but is not limited to them [Clark 1994, Foley et al. 1998]. In order to identify the parasite in the blood, a blood smear test is carried out by means of the Romanowski staining methods and the acridine orange method to stain bacterial DNA and RNA) as well as antibodies with fluorescein [Brinson 2001, Foley et. al. 1998, Westfall et al. 2001]. In the blood stained with the Giemsa method, the bacteria are an intense red colour, and they are blue-red if a Wright staining method is used. When observed through a light microscope, the bacteria have the forms of cocci, rings or bacilli stuck to the erythrocyte surfaces in clusters, pairs or as single bacterium, they often form chains in different places on erythrocytes [Small and Ristic 1967, Weiser 1981].

This parasitic bacterium possesses a double cell membrane, yet, it does not have a cell wall [Simpson et al. 1978, Woyciechowska and Frygin 1978].

In an electron microscope short bacilli, cocci and ring-chain forms can be observed. [Maede and Murat 1978, Weiser 1994] They have thin fibres which connect the parasite to the erythrocyte cell membrane. The parasite is located in a shallow depression formed in the erythrocyte cell membrane and is surrounded by folds. It does not penetrate the cell membrane of an erythrocyte, yet, it can erode its surface [Beaufils 2000, Demaree 1972, Jain and Keeton 1973].

FIA is a contagious disease transmitted with blood [Harbutt 1996]. Infection most often occurs through the biting of the animals by fleas, ticks or other blood sucking insects which were earlier to be found on an infected cat [Essex et al. 1975, Green 1998, Majda-Stanisławska 1997]. Infection can be transmitted during fights between cats or even during blood transfusions if blood is infected with the bacteria, or it can also be transmitted from an ill mother to her foetus or offsprings (the vertical way of the microbe transmission). For the latter, it is unclear if the infection occurs through the placenta during birth, or breast feeding and care from the mother [Adams et al. 1993, Ojeda and Skives 1978].

The aim of this study is to show that the performance of blood smear tests in suspected animals is useful for the FIA diagnosis.

MATERIAL AND METHODS

Samples for examination for FIA were taken from 20 cats which were brought to the "Legwan" Veterinary clinic with atypical disease symptoms in the years 2008–2009. If provide that occurrences of anaemia in the haematological investigation (haemoglobin less than 4 g/dl), as well as symptoms of apathy, emaciation, and an increase in body temperature were the criteria for the chosen cat. There were 20 cats, including 13 male cats (65% of the studied group) and 7 female cats (35% of the studied group) that met the criteria stated in the materials and methods sections assigned to the investigation. The group varied little in regard to age: 16 animals (80% of the studied group) were under 8 and the remaining 4 cats were (20% of the studied group) over 8 years of age. All animals in the group were crossbred cats. As many as 14 individuals (70% of the studied group) were untamed animals that went outside to a home garden or even much further than that, and 6 cats (30% of the studied group) were domestic cats.

Blood samples were taken three times every four days, and the blood collection was stopped each time the parasite was found in a blood smear. The blood smear tests were performed directly after blood collection (from fresh blood). In order to do so, the blood was smeared on a slide, which had been degreased thoroughly with 70% ethyl alcohol solution. Next, it was left to dry for 10-20 minutes after which it was stained with the use of the May-Grünwald-Giemsa method (M-G-G), [Harvey and Gaskin 1997, Jain and Keeton 1973]. As soon as the blood smear was dry, the preparation was observed under a light microscope with a magnification of 1x1000. Generally when this method is used, *Mycoplasma haemofelis* is stained dark blue [Christoper et al. 1990, Feldman et al. 2000, Jain and Keeton 1973, Łukaszewska et al. 2004, Taskier et al. 2001].

RESULTS

In blood smears, single or multiple spherical or bacillary shaped forms with a size of approximately 0.8-1.5 μm, which were located adjacent or close to the cell membrane of erythrocytes, were analyzed (Fig. 1).

FIA was found in 16 cats of the group studied (20 individuals were assigned to the group), which constitutes 80%. The first examination proved FIA in 8 animals (40%), the second proved the disease in 5 individuals (25%), and FIA was them identified in 3 individuals in the third examination (15%), (Tab. 1).

All the cats assigned for the studied had a lowered level of haemoglobin in blood which would suggests anaemia in these animals. The haematological results were not taken into consideration in this study as they came from different laboratories (clients of the veterinary surgery frequently brought the results of examinations carried out in other laboratories), and the examination was performed at a different time after the disease symptoms occurred (it was not always possible to determine the time of the disease unambiguously). The haematological results were only helpful for the classification of an animal for the studied group. As many as 16 individuals (80%) showed apathy characterized by an unwillingness to play or to go out, as well as sluggishness. Only 4 animals (20%) did not show very severe apathy, yet, this could be the result of the animal owner's poor observation. Similar results were obtained for the loss of appetite i.e. 16 animals consti-

tuting 80% of the studied group showed severe loss of appetite, and 14 animals (60%) clearly started to lose weight. In four cases, the animal owners reported that their cats ate slightly less than usual, however, as some of them claimed, this could be the result of the fact that the animals hunted, ate more or were fed outside. In the cases of 11 individuals (55%), the owners complained of fetor ex ore, and in 9 of them (45%), FIA was found. In 18 individuals (90%), a clear paleness of the mucous membrane was proved in the clinical examination, out of which 15 animals suffered from FIA. This constituted 75% of the studied group, whereas a distinct yellow colour in the oral cavity and the conjunctiva mucous membranes was observed in 14 individuals (70%), including 12 individuals (60%) suffering from FIA. Lymph nodes enlargement was found in 9 cats (45%). In 3 cases, the ultrasonic examination showed severe spleen enlargement, although only 8 animals were subject to the examination.

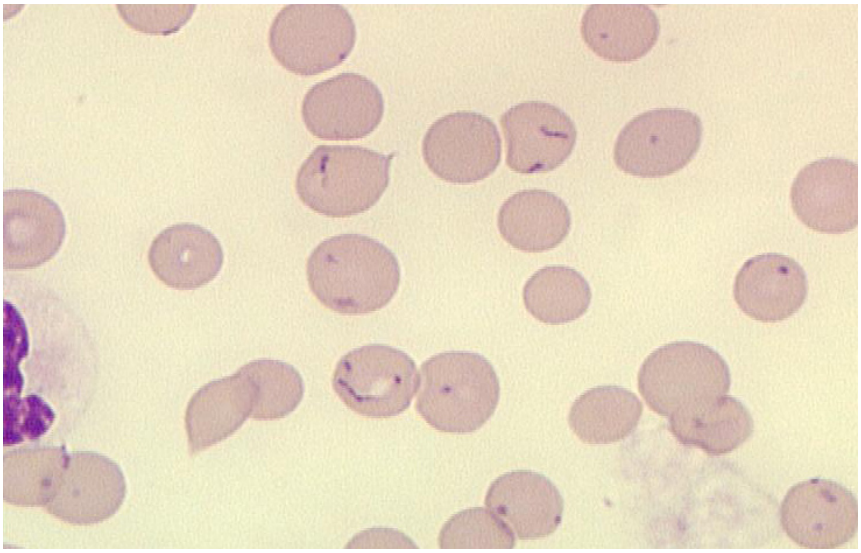

Fig. 1. *Mycoplasma haemofelis* cells can be seen on erythrocytes
Ryc. 1. Widoczne komórki *Mycoplasma haemofelis* na erytrocytach

Table 1. Results of examination for FIA with using of the blood film method in cats assigned for the studies
Tabela 1. Wyniki badań rozmazu krwi u kotów w kierunku obecności *Mycoplasma haemofilis* zakwalifikowanych do grupy badanej

Assigned for examination Zakwalifikowane do badań	Total number of cats in which *Mycoplasma haemofilis* was found: Łączna liczba kotów, u których stwierdzono *Mycoplasma haemofilis:*				
	First examination Pierwsze badanie	Second examination Drugie badanie	Third examination Trzecie badanie	Cats with positive smear test Koty z pozytywnym rozmazem	Cats with negative smear test Koty z negatywnym rozmazem
20	8	5	3	16	4
100%	40%	25%	15%	80%	20%

DISCUSSION

The study was carried out at the "Legwan Veterinary" Clinic located in Wrocław. 20 candidates that had previously meet the classification criteria were chosen among the cats brought to the clinic within the years 2008–2009. A drop in haemoglobin level below 4 g/dl, as well as clinical symptoms such as apathy, emaciation, and an increase in body temperature above 39.5°C were the criteria required for classification [Vensteenhouse et al. 1995, Weiser 1994]. The haematological analysis of the blood samples (the blood cell counting) were performed in specialised laboratories, whereas the clinical symptom outline was obtained from observation of the animal during examination as well as from an interview with the animal owner. Following the admittance of the animal to the study, a full blood smear test was carried out with the use of the M-G-G staining method. Out of 20 cats, 16 had *Mycoplasma haemofelis* bacteria in their blood smears, which constituted 80% of the studied group. In 8 animals (40%) the presence of bacteria was found during the first examination. In 5 cats during the second, and in only 3 cats during the third examination. The results proved other experimenters' observations [Łukaszewska et al. 2004], which consequently proved the presence of bacteria on the erythrocyte surface of the blood film. According to the above-mentioned, performing of the blood smear test just once does not guarantee a hundred percent FIA diagnosis as the bacterium is disseminated into the blood periodically [Harvey and Gaskin 1978]. Therefore blood smear tests should be carried out at intervals over several days a definite FIA diagnosis can be made. The results also stated that in 5 cats (which constitutes 25% of the studied group) the presence of *Mycoplasma haemofelis* was found only during the second investigation, whereas in 3 cats, (which constitutes 15% of the studied group) the presence of the bacteria was found only during the third investigation. Therefore, it seems to be necessary to perform a minimum of three blood smear tests every 4 days in order to definitely dismiss or exclude this disease entity. Infection can be passed on through the biting of bloodsucking ectoparasites (fleas, ticks) or even during fights between cats (through contact with blood). FIA very often occurs together with cat viral leukaemia (FeLV), especially in violence between untamed male cats [Feldman et al. 2000]. According to Łukaszewska et al. [2004], going outside increases the probability of contracting the FIA illness. The study comprised 15 cats suffering from FIA, including as many as 13 untamed cats and only 2 domesticated cats. Observations made during the investigation proved that untamed cats are more exposed to FIA infection than domesticated [Łukaszweska et al. 2004]. In our study out of 20 cats subjected to the study, 16 of them were ill. This included as many as 13 cats (which constitutes 65% of the studied group) which were untamed cats and only 3 domesticated cats (which constitutes 15% of the studied group). The results confirmed the previous observations made by Łukaszewska et al. [2004].

Out of the 16 cats in which the presence of *Mycoplasma haemofelis* was found, 12 individuals (which constitutes 60% of the studied group) were under 8 years of age, whereas only 4 individuals (which constitutes 20% of the studied group) were at the ages of 8 - 16. According to previous data, FIA occurs more often in young cats, between 1st and 3rd year of age [Chandler et al. 1994, Evans and Gruffydd-Jones 1984]. This is confirmed by our observations. Among 16 cats in which FIA was found, there were 9 male individuals and 7 females. Thus, it can be concluded that the sex of an animal does not have an influence on FIA frequency (statistically significant differences were not found between

a percentage of ill males and females in the examinated cats). Many suggest, however, that males are more predisposed to catching FIA than females [Chandler et al. 1994].

The level of apathy of an animal was one of the criteria taken into account during the investigation. All individuals with FIA in our study showed more or less relatively intense apathy. Cats that suffer from *Mycoplasma haemofelis* are depressed, weak, and have light pink (pale) gums and tongue [Clark et al. 2002]. A decrease in appetite was evident in all 16 ill animals, and, in addition, the cats showed a paleness or yellowish tinge in their mucous membranes, weakening, tachycardia, or tachypnoe [Kurzeja 1979, Dunn 1990]. According to Łukaszewska et al. [2004], the FIA infection is often accompanied by stomatitis. It is possible that it is connected to an occurrence of viral leukaemia FeLV in these animals [Łukaszewska et al. 2004]. Body mass loss occurs as a result of appetite decrease, which was observed in 14 individuals, which constituted 60% of the studied group.

Among the symptoms observed in the course of FIA and described in the available literature, the following should be mentioned: mucosal ischaemia, vomiting, repeated diarrhoeas, and icterus [Vensteenhouse et al. 1995]. Tachycardia, tachypnoea, hepatomegaly, and splenomegaly can also occur [Chandler et al. 1994, Harvay and Gaskin 1997]. In our study, mucosal ischaemia was found in 15 animals (which constituted 80% of the studied group) out of which only 3 animals were not infected with FIA, and a yellowish tinge of the mucous membranes was found in 12 ill individuals (which constituted 60% of the studied group). Thorough clinical examination of the animal, especially an observation of its oral cavity and conjunctiva mucous membranes is extremely useful for the FIA diagnosis [Chandler et al. 1994, Couto and Nelson 1992, Cowell et al. 1999]. The symptoms that accompany the *Mycoplasma haemofelis* infection are also fetor ex ore (found in 9 individuals, which constitutes 45% of the studied group), lymph nodes enlargement (found also in 9 ill individuals, which constitutes 45% of the studied group), and spleen enlargement (found in 3 individuals, which constitutes 15% of the studied group).

Fetor ex ore is connected with stomatitis caused by a simultaneous occurrence of Feline viral leukaemia [Łukaszewska et al. 2004]. However, the lymph nodes enlargement is associated with an inflammation process [Dunn 1990, Wightman 1980].

Performing of an ultrasound scan of the abdominal cavity is also useful for the FIA diagnosis. In the ultrasound scan, one can find the enlargement of the spleen, liver and mesenteric lymph nodes. However, these symptoms do not always occur during the course of the FIA disease, and the ultrasound scan examination can only be helpful if these symptoms are present, this is proved by the results obtained in our study, in which spleen enlargement was proved in 3 individuals (which constituted 20% of the studied group).

In the blood haematological investigation, the use of a fresh blood smear test is useful for the diagnosis of FIA, which has been proved in this paper.

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PRZYDATNOŚĆ ROZMAZÓW KRWI W DIAGNOZOWANIU ANEMII ZAKAŹNEJ KOTÓW

Streszczenie: Zakaźną anemię kotów (FIA – Feline infectious anemia) wywołuje drobnoustrój *Mycoplasma haemofelis*. W celu zdiagnozowania pasożyta we krwi wykonuje się rozmaz krwi barwiony metodami Romanowskiego lub przy użyciu oranżu akrydyny (barwienie DNA i RNA bakterii) oraz przeciwciał znaczonych fluoresceiną.
Celem badań było wykazanie przydatności wykonywania rozmazów krwi podejrzanych osobników w rozpoznawaniu FIA. Na podstawie wyników badań stwierdzono, że bazując jedynie na objawach klinicznych, nie można jednoznacznie postawić diagnozy w przypadku FIA, gdyż tylko u 80% kotów z podejrzeniem FIA odnotowano obecność bakterii na erytrocytach, mimo że objawy (apatia, spadek apetytu, utrata masy ciała, nieprzyjemny zapach z pyska, bladość błon śluzowych, zażółcenie błon śluzowych, powiększenie węzłów chłonnych, powiększenie śledziony) niejednokrotnie nasuwają podejrzenie choroby i potrafią ukierunkować lekarza. Wykonanie rozmazu krwi jest dobrą pomocniczą metodą diagnostyczną w przypadku FIA, ale powinno być wykonywane przynajmniej 3-krotnie w odstępach kilkudniowych.

Słowa kluczowe: zakaźna anemia kotów, FIA, *Mycoplasma haemofelis,* kot, rozmaz krwi

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