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EFFECT OF *CAMELINA SATIVA* OIL AND EXPELLER CAKE ON PLASMA LEVEL OF IODOTHYRONINES AND LIPID PROFILE OF BROILER CHICKENS

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The aim of the study was to determine the effect of camelina (Camelina sativa) oil or cake as dietary components for broiler chickens on the level of thyroid hormones, glucose, triglycerides and the level of cholesterol and its fractions in blood plasma. Chickens of the control group (I) were fed with a standard grower-type compound feed with 6% of rapeseed oil. The experimental groups received a diet containing 4% of cold pressed camelina oil, (Group II) or 10% camelina cake (Group III). On day 42 of life 8 chickens from each group were slaughtered. Blood was collected from the jugular vein to determine the level of blood plasma glucose, triglycerides, total cholesterol and its HDL fraction, as well as thyroid hormones. The introduction of camelina oil resulted in reduction of lipid ratios (triglycerides, total cholesterol and HDL and LDL fractions) in chicken plasma. Significantly lower (P <0.05) cholesterol and its HDL and LDL fractions were also observed in experimental groups II and III. Glucose content in the blood plasma of chickens from groups I and III was similar, and the difference did not show statistical significance. In contrast, in group II, a significantly lower (P<0.05) value of this parameter was observed compared to the control group. In the plasma of chickens from group III, fed with a diet containing camelina cake, a significantly higher (P<0.05) content of triiodothyronine (T3) was observed compared to the other groups. Group II, which received a 4% camelina oil mix showed the lowest (P<0.05) T3 level and the highest (P<0.05) T4 level in the blood plasma. The presented results indicate that camelina oil as well as expeller cake do not adversely affect the thyroid functions. Camelina oil decreased the level of cholesterol and its fraction as well as triglyceride and glucose levels.

Key words: broiler chicken, Camelina sativa, PUFA n-3, blood plasma, lipid profile, thyroid hormones

Modern broiler chickens are genetically focused on obtaining high production results. In order to achieve the full genetic potential, however, proper balance of the diet

is necessary. The fast growth rate requires ensuring high energy concentration in the feed, which will cover the demand of birds (Cherian, 2015; Ravindran et al., 2016). In order to achieve that, fat additives of animal origin, standardized fats or vegetable oils are used in compound feeds (Alagawany et al., 2019). Extensive research has shown that the amount and type of fat and its composition are important for obtaining good production results and maintaining the health of birds, which translates into obtained quality of meat (Panda et al., 2015; Alagawany et al., 2019). The best results can be obtained by feeding chickens with diets containing vegetable oils, which not only improve the digestibility of diet and increase the weight gain of chickens, but also have a positive effect on the functional and sensory quality of meat (Panda et al., 2015; Kalakuntla et al., 2017). The vegetable oils are a source of essential unsaturated fatty acids, particularly α-linolenic acid (C18:3 ALA) from the *n-3* group, and linoleic acid (C18:2 LA) from the *n-6* group, which are involved in many metabolic processes (Cherian, 2015). The deficiency of these acids and the abnormal proportions of *n-6/n-3* PUFA in the bird diet may result in a decrease in disease resistance, a reduction in body weight gain and increased fatness (Lee et al., 2019).

The effect of polyunsaturated fatty acids on the lipid metabolism of broiler chickens has been demonstrated (Ferrini et al., 2010; Head et al., 2019). Thyroxine (T4) and triiodothyronine (T3) are among the most important regulators of lipid metabolism in adipose tissue, as well as their concentration in blood plasma. These hormones affect the basic metabolism and thermogenic processes (Lachowicz et al., 2008); thyroid hormones exert anabolic or catabolic effects at low or high concentrations, respectively (Ferrini et al., 2010). In terms of lipid metabolism, iodothyronines have an effect on the synthesis, mobilization and degradation of lipids, although degradation not only affects synthesis (Darras et al., 2000). The most important actions of iodothyronines on lipid metabolism include: increased synthesis and mobilization of triglycerides stored in adipose tissue, increased concentration of non-esterified fatty acids, as well as increased lipoprotein lipase activity (Darras et al., 2000).

Camelina sativa is one of the richest known sources of α-linolenic acid of *n-3* group which is the precursor of longer derivatives like docosahexanenoic fatty acid (DHA) and eicosapentaenoic fatty acid (EPA) (Waraich et al., 2013). In cold pressed camelina oil, polyunsaturated fatty acids represent more than 50% of all fatty acids (Waraich et al., 2013). Camelina oil is a component that increased content of PUFA*n-3* in poultry products without adversely affecting the production parameters and meat quality (Aziza et al., 2010; Pietras and Orczewska-Dudek, 2013; Jaśkiewicz et al., 2014). Furthermore camelina oil improved sensory attributes of meat, especially juiciness and tastiness (Orczewska-Dudek and Pietras, 2019). Camelina product as an alternative and cheaper source of PUFA*n-3* in poultry diet has already been studied (Aziza et al., 2010; Pekel et al., 2015; Anca et al., 2019; Orczewska-Dudek and Pietras, 2019). Camelina cake, a by-product of oil production, became a focus of nutritional studies in animals due to fat content with high concentration of α-linolenic acid (Aziza et al., 2010; Meadus et al., 2014; Pekel et al., 2015) and protein content (even up to 45%) with beneficial amino acid composition (Aziza et al., 2010; Pekel et al., 2015). However, like a majority of plants belonging to this family, camelina contains glucosinolates which exert a disadvantageous effect on thyroid gland function, cause

hypertrophy of this gland and reduce T3 and T4 content in blood, principally due to the inhibition of iodine uptake by the thyroid gland (Clarke, 2010). In addition, they reduce feed consumption and suppress growth and fertility of animals, cause mucosal irritation in the gastrointestinal tract resulting in local necrotic foci (Clarke, 2010). Glucosinolate content in camelina cake ranges from 20.3 to 24.4 μmol/g (Pekel et al., 2009; Aziza et al., 2010b; Pekel et al., 2015) and is higher than that noted in rapeseed cake which ranges from 1.55 to 24 μmol/g (Thacker and Widyaratne, 2012). However, camelina cake contains fewer tannins and sinapine than rapeseed cake (Thacker and Widyaratne, 2012). Sinapine contents in camelina seeds vary between 5 and 17 μmol/kg, and in rapeseed from 22 to 41.8 μmol/kg (Matthäus and Zubr, 2000). Camelina seeds do not contain progoitrin and sinigrin (Matthäus and Zubr, 2000). Camelina cake contains the largest amounts of 10-methylsulfanyldecyl glucosinolate (glucocamelina), 9-methyl-sulfinylnonyl glucosinolate (glucoarabin) and 11-methylsulfinylundecyl glucosinolate (Pekel et al., 2009; Aziza et al., 2010; Pekel et al., 2015). These glucosinolates show anticancer properties (Berhow et al., 2013). Results of previous research showed that camelina meal or cake in amount of 100 g/kg in chicken broiler diet did not impair production performance and meat quality (Aziza et al., 2010; Pietras and Orczewska-Dudek, 2013; Pekel et al., 2009; Orczewska-Dudek and Pietras, 2019). In broiler chicken, there have been no studies on the concurrent measurement of plasma lipid and thyroid hormone levels after dietary supplementation with *Camelina sativa* oil or camelina cake.

Based on the above-mentioned data, the present studies were designed to determine the effect of *Camelina sativa* oil and cake as a source of *n-3* polyunsaturated fatty acids, in diets for broiler chickens on the plasma levels of thyroid hormones, glucose, triglycerides, cholesterol and its fractions.

Material and methods

Animal material, housing, feeding

The experiment was carried out according to the guidelines of the Ethics Committee for the Use of Animals in Research and no explicit approval of the Ethical Committee was needed because the animals were only fed different diets, none of them was toxic (Commission Regulation (EU) No. 68/2013 of 16 January 2013, allowing the use of *Camelina sativa* seeds and products derived thereof, including camelina oil and meal, as a feed component in animal diets) and no invasive procedures were applied to the chickens. The animals were sacrificed using methods adapted to the age, species and body weight of the animals and were carried out in accordance with the procedures described in Annex IV of Directive 2010/63/EU of 22 September 2010 on the protection of animals used for scientific purposes.

The experiment was performed on 486 Ross 308 broiler chickens (hens and cockerels) reared from 1 to 42 days of age in group pens on litter under standard environmental conditions with constant access to feed and water. In the first growth period, all birds were fed a starter diet. In the second growth period 22-day-old chickens were randomly assigned to 3 groups with 6 replications with 27 birds in each. Chickens in

the control group (I) received a standard grower diet containing 6% rapeseed oil. The experimental groups were fed with dietary 4% cold pressed oil of spring *Camelina sativa* var. Borowska (group II) or 10% camelina expeller of the same variety (group III). The experimental diets were fed for a period of 21 days. The feed mixtures used in the experiment were prepared according to the Nutritional Standards for Poultry (2005) and computed using WinPasze Pro (2006) software, taking into account the chemical composition of the experimental components used. The raw fibre in the experimental feed mixtures did not exceed 4%. Composition of feed mixture was presented earlier by Orczewska-Dudek and Pietras (2019). Nutritional value of the grower diets for broiler chickens is presented in Table 1.

Table 1. Nutritional value of grower-finisher diets

Component (%)	Group		
	RO	CO	CEC
Rapeseed oil	6.00	2.00	5.00
Camelina oil	–	4.00	–
Camelina cake	–	–	10.00
Contents of nutrients per 1 kg of feed mixture (%):			
metabolizable energy (MJ)	13.00		
total protein	20.00		
lysine	1.15		
methionine	0.52		
Ca	0.92		
P available	0.40		

Groups: RO – control 6% rapeseed oil, CO – 4% camelina oil and 2% rapeseed oil, CEC – 10% camelina cake and 5% rapeseed oil

Fatty acid profile and contents in feed mixtures were determined using a modified method of Loor and Herebein (2001), based on ISO 12966-2:2011. Fatty acids were separated and assayed in the form of methyl esters using a gas chromatograph VARIAN 3400 with a flame ionization detector (250°C, range = 11; carrier gas: helium, 3 ml/min; gas injection: 0.7 mcl), with the use of a capillary column Rtx 2330 measuring 105 m × 0.32 mm, 0.2 mm.

At the end of the experiment (day 42 of age), 8 birds from each group (4 cocks and 4 cockerels) were randomly selected and slaughtered using a method adapted to their age, species, and body weight. If no pain or suffering was inflicted during the trial, the regulations allowed sacrificing the experimental birds before sampling. The blood samples were taken from jugular vein after scarification, into tubes with heparin, then were centrifuged in MPW Med Instruments 6K15 centrifuge at +4°C for 15 min, 3000 rpm. The concentrations of glucose, triglycerides, total cholesterol and its HDL fraction were determined in plasma by enzymatic-colorimetric method using diagnostic kits manufactured by Pointe Scientific. The determinations were made in a Beckman spectrophotometric reader. LDL cholesterol content was calculated according to the Friedewald formula: LDL = total cholesterol – (HDL cholesterol + triglycerides/5). Thyroid hormones: ty-

roxine (T4) and triiodothyronine (T3) were determined with radioimmunoassay using commercial (RIA) kits (Diasource) according to procedures described in manual instructions, using the LKB Wallac minigamma reader.

Statistical analysis

The results were analyzed by including 8 replications per treatment, statistical analysis of the obtained indices was performed using a one-way analysis of variance. The significance of differences between the experimental groups was evaluated using the Duncan test. The differences were deemed statistically significant at a confidence level of $P<0.05$. The procedures were carried out using the SAS statistical package (version 9.2), procedure GLM.

Results

Supplementation of grower diet for broiler chickens with camelina oil or cake influenced profile of polyunsaturated fatty acids of these feed mixtures (Table 2).

Table 2. Fatty acid profile in feed mixtures for broiler chickens (% of total fatty acids)

Fatty acids	Group		
	RO	CO	CEC
SFA	19.21	16.14	11.78
UFA	80.79	83.86	88.22
MUFA	46.90	46.74	43.35
PUFA	33.89	37.12	44.87
PUFA*n-6*	9.23	24.40	31.99
PUFA*n-3*	1.35	11.59	12.65
PUFA*n-6/n-3*	6.82	2.10	2.53

Groups: RO – control 6% rapeseed oil, CO – 4% camelina oil and 2% rapeseed oil, CEC – 10% camelina cake and 5% rapeseed oil.

Table 3. Results of broiler chicken plasma analysis

Components	Group			SEM
	RO	CO	CSC	
Triglycerides (g/l)	0.46 a	0.32 b	0.40 ab	2.57
Cholesterol (g/l)	1.13 a	1.03 b	1.10 ab	2.87
%HDL	70.80 a	73.80 b	71.00 ab	2.11
LDL (g/l)	0.23 a	0.21 b	0.23 ab	1.98
LDL/HDL (g/l)	0.29	0.28	0.29	2.03
Glucose (g/l)	273.25 a	265.88 b	269.88 ab	2.66
T3 (nmol/l)	4.67 b	3.45 c	5.20 ab	0.41
T4 (nmol/l)	29.5 b	33.80 a	31.91 ab	2.27

A, B – mean values in a row marked with different letters vary significantly at $P<0.01$.

a, b, c – mean values in a row marked with different letters vary significantly at $P<0.05$.

Groups: RO – control 6% rapeseed oil, CO – 4% camelina oil and 2% rapeseed oil, CSC – 10% camelina cake and 5% rapeseed oil.

Addition of the test components caused reduction of saturated fatty acid (SFA) content from 19% in the control group to 16.1% and 11.7% in experimental groups and increased *n-3* PUFA content (from 1.5% to ca. 12%). Experimental feed mixtures were characterized by a narrow *n-6/n-3* PUFA ratio compared with the control mixture (6.8%).

The contents of analyzed parameters in plasma of broiler chickens are presented in Table 3. Supplementation of camelina oil to feed mixture significantly ($P<0.01$) reduced content of triacylogriceryde (by ca. 30%) in plasma of broiler chickens from group CO.

In experimental groups CO and CSC, also the contents of cholesterol and its HDL and LDL fractions were significantly reduced ($P<0.05$). Plasma glucose contents in group RO and CSC chickens were similar and the difference was not statistically significant. On the other hand, glucose content in group CO was significantly lower compared with the control group ($P<0.05$). Plasma triiodothyronine (T3) content was significantly higher in group CSC chickens fed the diet supplemented with camelina cake in comparison with the remaining groups ($P<0.05$). Group CO chickens receiving the diet containing 4% of camelina oil were characterized by the lowest T3 plasma level ($P<0.05$) and the highest T4 level ($P<0.05$).

Discussion

The results of earlier studies have shown that the camelina cake as a source of *n-3* PUFA in the broiler chickens diet at the level of 10% did not negatively impact growth performance and meat quality of broiler chickens (Orczewska-Dudek and Pietras, 2019). These results were in accordance with those described by Aziza et al. (2010) and Anca et al. (2019). In addition, a beneficial effect of 4% of camelina oil on meat juiciness and palatability was found. Camelina product as feed component in poultry diet has already been studied (Aziza et al., 2010; Pekel et al., 2015; Anca et al., 2019; Orczewska-Dudek and Pietras, 2019), but there is no data available about the effect of camelina cake or camelina oil on thyroxine and triiodothyronine plasma concentration in broiler chicken plasma.

Broiler chicken feed supplementation with 4% camelina oil induced a significant reduction of the plasma level of triglycerides and total cholesterol and its HDL and LDL fractions. The obtained results confirm the previous report by Pietras and Orczewska-Dudek (2013) indicating hypocholesterolemic effect of camelina oil. The authors demonstrated at that time that the addition of 6% camelina oil to feed mixture significantly reduced the plasma level of cholesterol and its HDL and LDL fractions but did not influence triglyceride level in chickens. Hypocholesterolemic properties of camelina oil were also confirmed by medical studies in humans. Administration of camelina oil at a dose of 35 ml/day for 2 weeks reduced plasma levels of total cholesterol and its LDL fraction (Karvonen et al., 2002). On the other hand, Eidhin et al. (2003) did not observe differences between plasma cholesterol content in pigs fed diet supplemented with 5% and 10% camelina oil for 3 weeks. Also Pietras and Orczewska-Dudek (2013) indicated that addition of 3% camelina oil to chicken diet did not

have such an effect on the analyzed parameters. However, according to Ciurescu et al. (2016), 2.5% camelina oil supplement in chicken diet reduced total cholesterol and its HDL and LDL fractions in plasma of broiler chickens and did not affect triglyceride level. Al-Shurbaji et al. (1991) suggested that PUFA showed hypolipidemic and hypocholesterolemic properties due to a strong suppression of lipid synthesis in the liver accompanied by the inhibition of synthesis of lipogenic and glycolytic enzymatic proteins, such as synthetase, acetyl-CoA carboxylase, 'malic' enzyme, glucokinase and L-pyruvate kinase. The mechanism of action of *n-3* PUFA on lipid and cholesterol metabolism is not completely understood. These acids can control transcriptional activity of nuclear receptors, thereby influencing the transcription of specific genes involved in lipid and carbohydrate metabolism (Jump, 2008). As already mentioned, the differences in the above-cited results obtained by different authors, regarding the effect of PUFA on plasma lipid levels can be the effect of different contents of *n-3* PUFA and *n-6* PUFA in camelina oil, dependent on the variety.

The present studies showed a tendency towards reduction of the plasma content of cholesterol and its fractions and triacyloglycerides in the chickens fed a feed mixture with 10% camelina cake. Anca et al. (2019) showed that feeding 8% camelina cake to broilers in second growth period, decreased significantly concentration of glucose, total choresterol and its LDL and HDL fractions. Taranu et al. (2014) observed a beneficial effect of camelina cake on reduction of cholesterol level in pig plasma. These authors also noted that camelina cake significantly reduced glucose level in plasma of these animals (by 18.47%), which was not noticed in the present studies. However, a significant reduction of plasma glucose concentration was seen in chickens receiving 4% camelina oil. Similar results were obtained by Taranu et al. (2014) when pig diet was supplemented with 12% camelina cake.

The *n-3* polyunsaturated fatty acids influence the blood level of thyroid hormones in birds (Ferrini et al., 2010). Thyroid hormones control basal metabolism (Abdel-Fattah et al., 1990) and are indispensable for proper growth and development of animals (Darras et al., 2000). These hormones also affect cholesterol metabolism and stimulate lipogenesis and oxidation of fatty acids (López et al., 2010). Souza et al. (2010) demonstrated that *n-3* PUFA could modulate lipid metabolism, partially by influencing the thyroid hormone activity in the liver via enhancing the hepatic expression of thyroid hormone receptor TRβ which plays a significant role in lipid metabolism in the liver. Studies of other authors also revealed that the type of fatty acids in fat influenced thyrotropin secretion, thyroid peroxidase and hepatic deiodinase activity (Lachowicz et al., 2008) and ability of T3 binding to nuclear receptors (Souza et al., 2010). Due to the presence of glucosinolates in camelina, in the presented experiment, the levels of thyroid hormones: triiodothyronine (T3) and thyroxine (T4) were also determined. In the presented experiment, broiler chickens fed the feed mixture containing 4% camelina oil showed reduced T3 level and elevated T4 level compared with the control group, which may suggest reduced T4 conversion to metabolically active T3. In contrast, Souza et al. (2010, 2011) considered that *n-3* PUFA supplied in the diet increased T4 conversion to T3. The mentioned authors used fish oil as a PUFA source, which contains higher concentrations of long-chain derivatives, like EPA and DHA. Similar results were obtained by Ferrini et al. (2010) with the use of 10% flax

oil in broiler chicken feeding. According to those authors feed supplementation with flax oil as an *n-3* PUFA source increased T3 concentration. They also suggested that PUFA could affect T3 level, thereby inducing changes in lipid metabolism expressed as a lower abdominal fat deposition in the carcass. Ketels and DeGroote (1989) demonstrated that elevated fat supply with the diet stimulated T4 conversion to T3. On the other hand, Navidshad et al. (2006) found that the type and amount of fat did not significantly affect plasma thyroid hormone level in broiler chickens. The action of thyroid hormones depends mostly on binding to nuclear receptors (Darras et al., 2011). Glucosinolates block thyroid hormone receptors and induce changes in the outer ring deiodination of T4 in peripheral tissues (Darras et al., 2000). As a result of this process, the concentration of T3 decreases and its rT3 form rises. It was found that camelina cake increased plasma T3 in chickens fed a mixture containing 10% of this component. The obtained results indicate an increased T4 conversion to T3 or to rT3. It could be caused by elevated content of erucic acid in camelina oil and camelina cake and thus its higher concentration in breast muscle lipids reaching 14%. However, Ryhänen et al. (2007) reported that the level of this acid in lipids of breast meat from chickens receiving feed supplemented with 10% camelina cake did not exceed 10%. The authors of the above-described studies did not observe the effect of camelina cake on the size of the thyroid gland in chickens. The newest studies of Moriel et al. (2011) did not show the effect of camelina expeller cake on hormone levels in the blood of cows, independently of the content of camelina expeller in feed mixture. In contrast, results of studies by Lardy and Kerley (1994) suggested that glucosinolates could affect the thyroid function which resulted in reduction of the contents of circulating T3 and T4. Based on experiments on broiler chickens, Ahmed et al. (2015) revealed that both 10% and 20% rapeseed cake supplement in feed mixture reduced T3 level. In the presented study, camelina cake at the level 10% slightly increased level of thyroid hormones but it was not confirmed statistically. This is in accordance with results of other authors which showed that camelina exceeding more than 10% affects growth performance and meat quality of broiler chicken (Aziza et al., 2010; Pekel et al., 2015). Glucosinolate concentration in camelina seeds is relatively low, however, their level depends on the variety, climatic zone and fertilization.

The introduction of dietary camelina oil has a significant effect on the levels of triglyceride, glucose and total cholesterol and cholesterol fractions. Feeding broiler chickens with camelina cake at 100 g/kg of diet in the second growth period had no negative impact on the lipid profile as well as on concentration of thyroxine and triiodothyronine in plasma. Camelina cake can be used in broiler chicken diet as a cheaper source of *n-3* polyunsaturated fatty acids at the level of 10%.

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Wpływ oleju i makuchu z lnianki siewnej (*Camelina sativa)* na poziom jodotyronin w osoczu i profil lipidowy kurcząt brojlerów

STRESZCZENIE

Celem badań było określenie wpływu oleju i makuchu z lnianki siewnej (*Camelina sativa*) w paszy dla kurcząt brojlerów na poziom ich hormonów tarczycy, glukozy, trójglicerydów oraz poziom cholesterolu i jego frakcji w osoczu krwi. Kurczęta z grupy kontrolnej (I) żywiono standardową mieszanką paszową typu grower z 6% udziałem oleju rzepakowego. Ptaki grup doświadczalnych otrzymywały mieszankę paszową dietę zawierającą 4% tłoczonego na zimno oleju z lnianki (grupa II) lub 10% wytłoków z lnianki (grupa III). W 42. dniu odchowu kurcząt z każdej grupy ubito po 8 ptaków. Krew pobierano z żyły jarzmowej, aby określić poziom glukozy w osoczu, trójglicerydów, cholesterolu całkowitego i jego frakcji HDL, a także hormonów tarczycy. Wprowadzenie oleju z lnianki spowodowało obniżenie wskaźników lipidowych (trójglicerydów, cholesterolu całkowitego oraz frakcji HDL i LDL) kurcząt brojlerów. W grupach doświadczalnych obserwowano także istotnie niższy (P<0,05). poziom cholesterolu i jego frakcji HDL i LDL. Zawartość glukozy w osoczu krwi kurcząt z grup I i III była podobna, a różnica nie wykazała istotności statystycznej. Natomiast w grupie II zaobserwowano znacznie niższą (P<0,05) wartość tego parametru w porównaniu z grupą kontrolną. W osoczu brojlerów z grupy III, żywionych dietą z udziałem wytłoków z lnianki, zaobserwowano znacznie wyższą (P<0,05) zawartość trijodotyroniny (T3) w porównaniu z innymi grupami. Grupa II, która otrzymała 4% oleju z lnianki, wykazała najniższy (P<0,05) poziom T3 i najwyższy (P<0,05) poziom T4 w osoczu krwi. Przedstawione wyniki wskazują, że olej i makuch z lnianki nie wpływają niekorzystnie na funkcje tarczycy. Olej z lnianki obniżył poziom cholesterolu i jego frakcji, a także poziom trójglicerydów i glukozy.

Słowa kluczowe: brojlery, *Camelina sativa*, PUFA *n-3*, osocze krwi, profil lipidowy, hormony tarczycy