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Original Article

Performance Parameters, Plasma Lipid Status, and Lymphoid Tissue Fatty Acid Profile of Broiler Chicks Fed Camelina Cake

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ABSTRACT

The effects of dietary camelina cake (CAMC) on broiler chicks'growth performance, plasma lipid status and fatty acid profile (FA) of lymphoid organs were evaluated. Six hundred broilers (3-week-old, Cobb 500) were randomly allotted for 20 days in a feeding trials into 2 groups: control (C; corn-soybean meal-canola meal basal diet) and experimental (CAMC; 80 g CAMC/kg diet replaces canola meal from C diet). Blood samples (n=12/group) were collected on day 42 for plasma profile analysis (glucose, Glu; total cholesterol, TC; high-density lipoprotein cholesterol, HDL-C; low-density lipoprotein cholesterol, LDL-C; triglycerides) and immune organs (spleen, thymus, bursa of Fabricius) for FA analysis. The FA profile of lymphoid tissue was determined by gas chromatography. Feeding the CAMC diet did not influence broilers performance parameters or relative weights of lymphoid tissue, except the weight of bursa that decreased (p<0.05). Plasma lipids profile was affected by decreasing (p<0.05) the concentrations of Glu, TC, HDL-C and LDL-C in broilers fed the CAMC diet. In all lymphoid tissue, the total content of n-3 polyunsaturated fatty acids (PUFA) increased (p<0.001) and the total level of n-6 PUFA decreased (p<0.001) as the effect of the CAMC diet. The n-6:n-3 ratio decreased (p<0.001) up to 5:1 in all lymphoid tissue. The higher (p<0.05) concentration of arachidonic acid was found in the spleen, followed by the thymus and the bursa of the chicks fed the CAMC diet. Our results indicate that feeding 80 g/kg camelina cakes in broiler chicks finisher diet had no negative impact on productivity, beneficially alter the plasma lipid status and fatty acids profile of lymphoid tissue.

INTRODUCTION

Over the last years, there has been an increased interest to modulate the n-3 polyunsaturated fatty acids (PUFA) profile of poultry meat in order to improve its nutritional value and to increase the consumption of these fatty acidsby humansdue to healthier benefits (Rymer & Givens, 2005; Wood *et al*., 2008; Poureslami *et al*., 2010; Ribeiro *et al*., 2013; Bhalerao *et al*., 2014; Nain *et al*., 2015).

Moreover, feeding n-3 PUFA enriched diets resulted in the deposition of these fatty acids into the lipid membrane of all tissues, including the cells of the immune system (Cinader *et al*., 1983; Huang & Fritsche, 1992) and could affect the immune response and reduce inflammation in different species such as chicken, mice and fish (Babu *et al*., 2005; Puthpongsiriporn & Scheideler, 2005; Calder, 2006; Wall *et al*., 2010). It is known that lymphoid tissues play an important role in the body defences against pathogens and its development can in some cases reflect immune system response and functionality (Grasman, 2002; Smith & Hunt, 2004; Akter *et al.*, 2006). In the

chickens' central lymphoid organs are the thymus and the bursa of Fabricius, while peripheral lymphoid organs include the spleen and all the lymphoid tissue associated with the intestinal mucosa. The spleen is the major site of immune responses to blood-borne antigens and is also a site of hematopoiesis (Batista & Harwood, 2009).

However, the potential to alter the nutrient composition of tissue strongly differs according to the feed ingredient considered. Recently, the development of the biofuels industry resulted in a significant increase of by-products (meals or cakes), vegetable sources rich in protein and energy which can be utilized in poultry diets.

Camelina oil cake (CAMC), a by-product of *Camelina sativa* (L. Crantz) oil seed plant, belonging to the *Brassica* family (Ghamkhar *et al*., 2010), has attained interest as a potential feed ingredient due to its high oil content (10 to 22%) and favourable FA composition, especially theα-linolenic acid content (16.28 to 29%; Cherian, 2012). The available scientific information on the effects of camelina oil cake or meal in broiler diets are conflicting regarding the production performance (Ryhanen *et al.,* 2007; Frame *et al*., 2007; Pekel *et al*., 2009; Pekel *et al*., 2015), or limited on fatty acid composition of muscle tissues (Cherian *et al.,* 2009; Aziza *et al.,* 2010; Cherian, 2012; Nain *et al.,* 2015) or other tissue such as liver and brain (Nain *et al.,* 2015).

Toour knowledge, there is no data available about the effects of CAMC, as a rich source of n-3 PUFA, on the plasma lipid status and fatty acid profile of lymphoid tissue in broiler chicks. Therefore, the aim of this study was to determine the effect of camelina cakes on performance, plasma lipid profile and lymphoid tissue fatty acid profile in broiler chicks.

MATERIAL AND METHODS

The study was performed at the experimental unit of the National Research-Development Institute for Animal Biology and Nutrition (Balotesti, Romania) based on a protocol approved by the Ethical Committee of the institute, in accordance with the EU Directive 2010/63/EU (OJEU, 2010).

Broilers, experimental diets, and sampling

Six hundred 3-week-old mixed-sex Cobb 500 broilers (883.26±15.04g) were used in a 20-day feeding trial (finisher phase, 23 to 42 days) conducted in a controlled experimental house. Birds were randomly allotted to 2 dietary groups, with 4 replicates of 75 broilers each and were raised in wood shaving-floor pens. Each pen was equipped with manual feeders and nipple drinker lines. A lighting program of 23hL:1hD was used for the experimental period. The birds had *ad libitum* access to feed and water.

The broilers were fed with a control diet based on a corn-soybean meal-canola meal (C) and an experimental diet containing 80 g/kg camelina cakes (CAMC) that replaced the canola meal from C diet. The finisher diets (Table 1) were isocaloric, isonitrogenous and formulated to meet the nutrient requirements of broiler hybrid (Cobb-Vantress,2015).The camelina cake used in this study was provided from the local commercial oil processing plant and was obtained by cold-pressing oil extraction method. The chemical composition of CAMC was 93.5% dry matter, 30.4% CP, 22.5% crude fat, 8.8% crude fibre, 7.4% ash, 0.5% calcium, 0.75% phosphorus, and 12.76 MJ/kg metabolisable energy. The protein of CAMC had a higher content of essential amino acids such a sarginine (2.85%), lysine (1.54%), threonine (1.1%), methionine (0.83%) and cysteine (0.71%).

The ingredients and analyzed composition of the experimental diets are given in Table 1.

Body weight (BW) and feed intake (FI) were measured and body weight gain (BWG), feed conversion ratio (FCR) was calculated from 23 to 42 days. Mortality rates were recorded daily, to make corrections in calculating FI and FCR during the experimental period.

At slaughter age (42 days), twelve birds per treatment (six male and six female) were randomly allocated for blood sampling and immune organs evaluation over a 12-h feed withdrawal period.

Blood samples (4 ml) were collected from wing vein in heparinized tubes, stored on ice and immediately processed. After blood sampling broilers were killed by cervical dislocation and bleeding. Carcasses were manually eviscerated and spleen, thymus and bursa of Fabricius were aseptically removed. Lymphoid organs were weighed and stored at -20°C until fatty acid analyses; their relative weights were calculated as the percent of live BW at slaughter.

Chemical and biochemical analysis

The chemical composition of the ingredients and the diets samples were analysed based on standard procedures (OJEU, 2009), in duplicate for: dry matter (SR ISO 6496:2001), crude protein (SR EN ISO 5983-2:2009 AOAC 2001.11), crude fat (SR ISO 6492:2001), crude fibre (SR EN ISO 6865:2002),

Table 1 – Ingredients and analyzed composition of diets.

Ingredients, g/kg, as-fed basis	Finisher phase, 23 to 42 days	
	C[1]	CAMC[2]
Corn	613.5	617.8
Soybean meal	178.0	188.3
Camelina cake	-	80.0
Canola meal	80.0	-
Corn gluten meal	25.0	25.0
Vegetable oil	54.0	39.0
Monocalcium phosphate	16.7	16.5
Calcium carbonate	14.6	14.9
Salt	3.0	3.0
Vitamin-mineral premix[3]	10.0	10.0
DL-Methionine	1.7	1.7
L-Lysine HCl	2.9	3.2
Choline HCl	0.6	0.6
Analyzed composition		
Crude protein	181.1	181.0
Lysine	10.56	10.54
Methionine+cystine	8.18	8.16
Calcium	8.8	8.9
Phosphorus	8.2	8.3
Crude fibre	37.8	38.0
Crude fat	62.8	63.8
Metabolisable energy (MJ/kg)[4]	13.39	13.39

[1]C - Control; [2]CAMC - Camelina cake; [3]Supplied per kg diet: retinyl acetate, 2.90 mg; cholecalciferol, 0.12 mg; DL-α-tocopheryl acetate, 50 mg; menadione sodium bisulphite, 3 mg; thiamine mononitrate, 2 mg; riboflavin, 8 mg; pyridoxine-HCl, 3 mg; cyanocobalamin, 0.015 mg; Ca-panthotenate, 12 mg; niacin, 50 mg; folic acid, 1.5 mg; Mn, 100 mg; Zn, 100 mg; Fe, 40 mg; Cu, 15 mg; I, 1.0 mg; Se, 0.30 mg; Co, 0.25 mg. [4]based on regression equations(NRC, 1994).

crude ash (SR EN ISO 2171:2010),calcium (SR ISO 6490-2:1983), phosphorus (spectrophotometry method) and amino acid profile (HPLC-high performance liquid chromatography). Metabolisable energy (ME) content was calculated based on the energy content of feed ingredients using regression equations (NRC, 1994).

Fatty acid profile of ingredients, diets and immune tissue samples were determined by the gas chromatography method (SR CEN ISO/TS 17764-2:2008) using Perkin Elmer500 chromatograph. The method was previously described by Hăbeanu *et al*. (2014) and consisted in transforming the sample fatty acids in methyl esters, followed by the separation of the components in the chromatography column, their identification by comparison with standard chromatograms and the quantitative determination of the fatty acids (expressed as % total fatty acid methyl esters-FAME).

Blood samples were centrifuged at 2,700 rpm for 10 min at 4°C, then separated plasma was transferred to Eppendorf tube and stored at -20°C until analyses. Plasma lipid profile (glucose, Glu; total cholesterol, TC; high-density lipoprotein cholesterol, HDL-C; low-density lipoprotein cholesterol, LDL-C; triglycerides, TG) were determined on chemistry analyzer (BS-130, Shenzhen Mindray Bio-Medical Electronics Co., China), using Accent-200 MG kits (Cormay, Wiosenna, Poland). Very low-density lipoprotein–cholesterol (VLDL-C) values were calculated by the formula: VLDL-C = Triglycerides divided by 5 (Tietz, 1996).

Statistical analysis

For performance parameters, replication was considered as the experimental unit for the statistical analysis and other results were analyzed with every broiler as a replicate. Data were analyzed with a one-way ANOVA procedure of SPSS version 20.0 software (IBM SPSS Inc., 2014). The results were expressed as means with standard error of the mean (SEM) and value for fatty acids are expressed as the percentage of total fatty acid ester methyl (% of total FAME). Differences between means were considered statistically different at $p<0.05$.

RESULTS AND DISCUSSION

Fatty acid profile of camelina cake and diets.

Table 2 shows that the fatty acid profile of camelina cake used in our study and obtained by cold-pressing oil extraction method, is a rich source of α-linolenic acid (ALA, 29.47%), linoleic acid (LA, 21.09%), and oleic acid (17.69%).Similar results have been reported previously by Nain *et al.* (2015) and Juodka *et al*. (2018). The fatty acid composition of diets showed that the inclusion of 80 g/kg CAMC increased the ALA content (2.87-fold) and resulted in a 3.89-fold decrease of the LA: ALA ratio, compared with the classical diet. It is stated that ALA is the precursor of long-chain n-3 PUFA such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have received considerable attention as functional nutrients for their various health-promoting effects (Rymer & Givens, 2005; Schmitz & Ecker, 2008). Thus, to obtain an efficient conversion of ALA into EPA and DHA it is important to assure an adequate dietary LA: ALA ratio (Griffin, 2008).

Table 2 – Fatty acid profile (% of total FAME) of camelina cake and experimental diets.

Fatty acid (% of total FAME)*	Camelina cake	Finisher phase, 23 to 42 days	
		C	CAMC
C14:0 (myristic)	0.15	0.09	0.10
C16:0 (palmitic)	7.43	8.76	8.77
C16:1 (palmitoleic)	0.24	0.21	0.24
C18:0 (stearic)	2.01	2.87	2.38
C18:1cis-9 (oleic)	17.69	24.80	19.92
C18:2n-6 (linoleic, LA)	21.09	61.31	45.37
C18:3n-3 (alpha-linolenic, ALA)	29.47	2.24	6.44
ΣSFA	9.59	11.72	11.25
ΣMUFA	17.93	25.01	20.16
LA:ALA	0.72	27.37	7.04

C – Control; CAMC – Camelina cake; *FAME- fatty acids methyl esters; ΣSFA = C14:0 + C16:0 + C18:0; ΣMUFA = C16:1 + C18:1cis-9.

Performance parameters

The effects of dietary CAMC on production parameters and lymphoid tissue weights of broiler chicken are shown in Table 3. There was no significant difference (p>0.05) in BW, WG, FI and FCR between the dietary treatments at the end of the finisher phase (42 days). Similarly, previous studies feeding diets up to 10% camelina meal reported no significant differences in turkey broiler performance (Frame *et al.,* 2007) or in broiler final body weight, feed efficiency and carcass weight at 42 days of age (Aziza *et al.,* 2010). Other studies also indicated that the addition of camelina oil in broiler chicken's diets had no significant effects on performance and carcass quality parameters (Pietras & Orczewska-Dudek, 2013; Jaskiewicz *et al.,* 2014). On the contrary, Thacker & Widyaratne (2012) stated that dietary camelina meal inclusion up to15% decreased BW gain and impaired the feed conversion of broiler chickens. Pekel *et al.* (2015) fed broilers with 10 and 20% camelina meal from 21-28 days of age and reported detrimental effects on performance and attributed this negative impact to the presence of glucosinolates in camelina by-product.

In our study, the relative weights of lymphoidorgans at 42 days (Table 3) were not affected (p>0.05) by the CAMC addition in the diet, except the weight of the bursa of Fabricius that significantly decreased (p=0.039). Previous studies have shown that lymphoid tissue development can reflect the immune status (Grasman, 2002; Smith & Hunt, 2004; Akter *et al.,* 2006). Wang *et al*. (2000) reported that feeding laying hens with diets rich in n-3 PUFA stimulates the growth of immune tissue (thymus, spleen, and bursa) up to 4 weeks of age. The authors suggested that after 4 weeks of age the immune tissue weights began to decrease, and the bursa degenerated between the 4th and the 8th weeks of age. Al–Khalifa *et al.* (2012) have shown that feeding n-3 PUFA rich diets (30, 50 and 60 g/kg fish oil) had no effect on the relative weight of the spleen, but significantly higher the thymus weights in broilers fed diet with 50 g/kg fish oil and significantly lower the bursa weights in broilers fed diets containing 50 and 60 g/kg fish oil than those of broilers fed the control diet or 30 g/kg fish oil.

Table 3 – Effect of dietary camelina cake on broilergrowth performance, lymphoid organ weights and abdominal fat weight.

Item	Diets		SEM	p-value
	C	CAMC		
Finisher phase, 23 to 42 days[1]				
Initial body weight(g/broiler)	890.02	876.50	5.31	*0.228*
Final body weight(g/broiler)	2375.82	2340.13	38.01	*0.674*
Weight gain (g/broiler)	1485.80	1463.63	40.03	*0.805*
Feed intake (g/broiler)	2657.50	2640.00	14.12	*0.588*
Feed conversion ratio(g feed:g gain)	1.79	1.80	0.04	*0.867*
Lymphoid tissue, % of BW[2]				
Spleen	0.12	0.10	0.002	*0.723*
Thymus	0.20	0.17	0.01	*0.240*
Bursa of Fabricius	0.18^{a}	0.13^{b}	0.004	*0.039*
Abdominal fat, % of BW[2]	1.90	1.86	0.07	*0.691*

C – Control; CAMC – Camelina cake; SEM – standard error of the mean;

[1]Means of 4 replicates per treatment (n = 75 broilers from each replicate), at 42 days of age. [2]Means of 12 broilers per treatment, at 42 days of age.

[ab]Means within a row with no common superscript are significantly different (p<0.05).

Plasma lipid status

The results indicated that dietary CAMC affects the plasma lipid profile (Table 4). Thus, glucose concentration was significantly decreased (4.37%; p=0.037), total cholesterol and its fractions HDL-C and LDL-C were also decreasedby12.30% (p=0.013), 9.21% (p=0.019) and 29.45% (p=0.002), respectively. The plasma LDL-C was negatively correlated with the ALA fatty acid content

of the diet (r= - 0.58, p=0.002). The values of very low-density lipoprotein cholesterol (VLDL-C) and triglycerides were lower compared to the C group with no significant difference (p>0.05). It is considered that VLDL-C concentrations are good indicators of fat deposition in the bird (Whitehead & Griffin, 1984; Grunder *et al.* 1987). This confirms the results of our study regarding the abdominal fat content, expressed as % of BW at slaughter, which was lower (1.86% *vs.* 1.90%; p>0.05; Table 3) compared to the C group as the effect of CAMC addition.

Our results are in line with what was previously reported by Taranu *et al.* (2014) who found that feeding camelina cake in finishing pigs improves the blood biochemistry profile by decreasing plasma glucose and increasing plasma antioxidant capacity. Fébel *et al.* (2008) have shown that feeding broilers with different sources rich in PUFA decrease plasma total cholesterol, and the decrease could be attributed to a suppression of hepatic cholesterol production by the high PUFA levels.

Table 4 – Effect of dietary camelina cake on the plasma lipid profile of broilers at 42 days.

Parameter (mg/dl)	Diets		SEM	*p*-value
	C	CAMC		
Glucose	271.06[a]	259.22[b]	2.87	*0.037*
Total Cholesterol	108.18[a]	94.87[b]	2.78	*0.013*
HDL-Cholesterol	72.33[a]	65.67[b]	1.75	*0.019*
LDL-Cholesterol	26.21[a]	18.49[b]	1.36	*0.002*
VLDL-Cholesterol	9.64	8.72	0.33	*0.170*
Triglycerides	48.19	43.58	1.67	*0.175*

C – Control; CAMC – Camelina cake; SEM – standard error of the mean;

VLDL-Cholesterol=Triglycerides divided by 5 (Tietz, 1996).

Means of 12 broilers per treatment, at 42 days of age.

[ab]Means within a row with no common superscript are significantly different (p<0.05).

Lymphoid tissue fatty acid profile

The effects of dietary CAMC on the fatty acid profile of the spleen, the thymus, and the bursa of Fabricius in broiler chicks are presented in Table 5. As response to feeding CAMC in broilers the total saturated fatty acids (SFA) was increased (p=0.029) by 1.08-fold in the thymus and 1.06-fold in the bursa, while the predominant SFA, palmitic acid (C16:0) increased in all immune tissues (1.06-fold in the bursa, 1.07-fold in the spleen and 1.09-fold in the thymus; p=0.002). The content of palmitoleic acid (C16:1) increased in all lymphoid tissues (1.33-fold in the spleen and in the bursa, 1.40-fold in the thymus; p<0.0001). However, the content of oleic acid (C18:1cis-9) decreased in the thymus and in the bursa (1.11-fold; p=0.009). Villaverde *et al.* (2006) reported that feeding broilers with rich PUFA diets could decrease the oleic acid due to an inhibition effect of increased dietary n-3 PUFAs on the Δ9-desaturase enzyme, lowering *de novo* synthesis of monounsaturated fatty acids (MUFAs).

Our results have shown that the MUFAs concentration significantly decreased (p<0.0001) in the thymus (1.04-fold) and in the bursa (1.07-fold) tissues as an effect of dietary CAMC addition. It is stated that the increase of PUFAs concentration led to decreases in MUFAs in tissues due to their inhibitory role of the desaturase enzyme needed for synthesis of MUFAs (Lefevre *et al.,* 2001).

As expected, the composition of the CAMC diet, especially the essential FA content is reflected in the PUFA profile of lymphoid organs. The LA (C18:2n-6) content as predominant n-6 PUFA significantly decreased in all tissues (1.58-fold in the bursa, 1.61-fold in the thymus and 1.30-fold in the spleen; p<0.0001) of broilers fed CAMC diet and the total PUFAs content also decreased (p<0.0001) compared with C diet. However, the increase in ALA (C18:3n-3) content (4.33-fold in the bursa, 4.29-fold in the thymus and 6.06-fold in the spleen; p<0.0001) led to an increase of the total n-3 PUFAs in all the immune tissues (4.0-fold in the spleen, 3.24-fold in the thymus and 3.29-fold in the bursa; p<0.0001) as effect of feeding CAMC diet.

Eicosadienoic acid (C20:2n-6) level was higher (p<0.0001) in all tissues (2.19-fold in the bursa, 3.62-fold in the spleen and 4.58-fold in the thymus) and eicosatrienoic acid (C20:3n-3) was lower (1.44-fold in the thymus, 1.63-fold in the spleen and 2.58-fold in the bursa; p=0.003) affected by dietary treatments. Arachidonic acid (AA; C20:4n-6) concentration signifi-cantly decreases (p=0.002) by1.32-fold in the thymus, 1.47-fold in the bursa and 1.78-fold in the the spleen.

Table 5 – Effect of dietary camelina cake on immune tissue fatty acid profile of broilers.

Tissue	Spleen		Thymus		Bursa Fabricius		SEM	*p*-value	
Diets	C	CAMC	C	CAMC	C	CAMC		Diet	Tissue
Fatty acids (% of total FAME)[1]									
C14:0	0.65	0.66	0.70	0.79	0.68	0.86	0.01	0.103	0.207
C16:0	$21.04^{b,z}$	$22.47^{a,z}$	$23.80^{b,x}$	$25.99^{a,x}$	$21.39^{b,y}$	$22.53^{a,y}$	0.31	0.002	<0.0001
C16:1	$4.07^{b,y}$	$5.40^{a,y}$	$4.28^{b,x}$	$5.99^{a,x}$	$3.41^{b,z}$	$4.53^{a,z}$	0.16	<0.0001	0.001
C18:0	8.75	7.46	6.62	6.69	8.43	9.00	0.20	0.532	0.158
C18:1cis-9	32.93^{a}	31.50^{b}	35.14^{a}	31.68^{b}	35.34^{a}	31.80^{b}	0.31	0.009	0.342
C18:2n-6	24.29^{a}	18.6^{b}	26.81^{a}	16.64^{b}	26.62^{a}	16.87^{b}	0.75	<0.0001	0.972
C18:3n-3	$0.86^{b,x}$	$5.22^{a,x}$	$0.82^{b,z}$	$3.52^{a,z}$	$0.99^{b,y}$	$4.29^{a,y}$	0.01	<0.0001	0.044
C20:2n-6	0.40^{b}	1.45^{a}	0.43^{b}	1.97^{a}	0.57^{b}	1.25^{a}	0.08	<0.0001	0.054
C20:3n-3	$0.62^{a,x}$	$0.38^{b,x}$	$0.36^{a,z}$	$0.25^{b,z}$	$0.49^{a,y}$	$0.19^{b,y}$	0.04	0.003	0.013
C20:4n-6	$3.76^{a,x}$	$2.11^{b,x}$	$2.71^{a,y}$	$2.05^{b,y}$	$1.16^{a,z}$	$0.79^{b,z}$	0.17	0.002	<0.0001
C20:5n-3	0.08^{b}	0.55^{a}	0.13^{b}	0.47^{a}	0.10^{b}	0.48^{a}	0.04	<0.0001	0.791
ΣSFA	30.52^{a}	30.78^{a}	31.44^{b}	33.80^{a}	30.60^{b}	32.45^{a}	0.35	0.029	0.059
ΣMUFA	37.97^{a}	37.68^{a}	40.02^{a}	38.35^{b}	39.38^{a}	36.85^{b}	0.41	<0.0001	0.328
ΣPUFA	30.01^{a}	28.33^{b}	31.26^{a}	24.90^{b}	29.93^{a}	23.87^{b}	0.67	<0.0001	0.175
Σn-6 PUFA	28.48^{a}	22.18^{b}	29.95^{a}	20.66^{b}	28.35^{a}	18.91^{b}	0.72	<0.0001	0.419
Σn-3 PUFA	$1.56^{b,x}$	$6.15^{a,x}$	$1.31^{b,z}$	$4.24^{a,z}$	$1.58^{b,y}$	$4.96^{a,y}$	0.26	<0.0001	0.017
ΣLCn-3 PUFA	0.70^{b}	0.93^{a}	0.49^{b}	0.72^{a}	0.59^{a}	0.67^{a}	0.05	0.047	0.093
Σn-6:Σn-3 ratio	18.25^{a}	3.61^{b}	22.86^{a}	4.87^{b}	17.94^{a}	3.81^{b}	1.28	<0.0001	0.144

C – Control; CAMC – Camelina cake; SEM – standard error of the mean; [1]FAME- fatty acids methyl esters.

Means of 12 broilers per treatment, at 42 days of age.

[ab] Means within rows with no common superscript are significantly different ($p<0.05$) for diet effect.

[xyz]Means within rows with no common superscript are significantly different ($p<0.05$) for tissue effect.

ΣSFA = C14:0 + C15:0 + C16:0 + C17:0 + C18:0; ΣMUFA = C15:1 + C16:1 + C17:1 + C18:1cis-9; ΣPUFA = C18:2n-6 + C18:3n-3 + C20:2n-6 + C20:3n-3 + C20:4n-6 + C20:5n-3; ΣLCn-3 PUFA = C20:3n-3 + C20:5n-3.

Eicosapentaenoic acid (EPA; C20:5n-3) content increased ($p<0.0001$) in the immune organs of broilers fed CAMC diet compared with C (3.62-fold in the thymus, 4.8-fold in the bursa and 6.88-fold in the spleen). It was noticed that the spleen, that is the major immune organ, contained more EPA than other immune tissues and this fact could be related with a higher capacity for eicosanoid production, which is predominated by arachidonic acid (Al-Khalifa *et al.,* 2012). The AA and EPA are metabolites of LA and ALA (Schmitz & Ecker, 2008) and usually AA decrease when n-3 PUFA increases (Komprda *et al.* 2005).

The sum of n-6 PUFAs decreased ($p<0.0001$) in the bursa (1.51-fold), thymus (1.45-fold), and spleen (1.28-fold) compared with the C diet. These results are in line with previous studies that stated that the LA and ALA compete for the same desaturase enzymes (Crespo & Esteve-Garcia, 2001; Lopez-Ferrer *et al.,* 2001).

As results of dietary CAMC inclusion, the n-6:n-3 ratio decreased in all immune tissues (4.87% in the thymus, 3.81% in the bursa and 3.61% in the spleen; $p<0.0001$) compared with C diet. It is stated that a lower ratio of n-6:n-3 PUFAs in poultry diets decreases the competition of ALA with LA for enzymes involved in bioconversion to long-chain (LC) n-3 PUFAs, resulting in increased tissue content (Riediger *et al*., 2009; Nain *et al*., 2012). In our study, the higher deposition of sum LCn-3 PUFAs was noticed in all immune tissue, especially in the thymus and the spleen ($p=0.047$) as an effect of dietary CAMC addition.

Regarding the tissue effects (Table 5) at the level of individual FA the significantly higher deposition was found for palmitic acid (C16:0)thymus > bursa >spleen ($p<0.0001$), palmitoleic acid (C16:1) thymus > spleen >bursa ($p<0.001$), ALA (C18:3n-3) spleen >bursa >thymus ($p=0.044$), eicosatrienoic acid (C20:3n-3) spleen >bursa >thymus ($p=0.013$), AA (C20:4n-6) spleen >thymus >bursa ($p<0.0001$). Total n-3 PUFA amount was significantly higher in spleen >bursa >thymus ($p=0.017$). Poureslami *et al.* (2010) have also reported differences in the tissue-specific distribution of n-3 PUFAs in broilers.

To our knowledge, there are no published reports evaluating the effect of dietary camelinacakes on the lymphoid tissue fatty acid profile. However, Al-Khalifa *et al.* (2012) studied the effect of feeding increasing levels of fish oil (30, 50 and 60g/kg) on immune function in Ross 308 broilers from 21 to 47days. These authors reported no significant effect of fish oil enriched diets on the proportions of ALA in the bursa; a significant

decrease in the total amount of n-6 PUFA, AA, and the n-6:n-3 ratio in the bursa, spleen and thymus concomitant with a significant increase of n-3 PUFA in immune tissue and blood leukocytes compared with the control diet. Recently, Nain *et al.* (2015) investigated the effects of different levels of dietary camelina cake (8, 16 and 24%) on lipid deposition in different tissue (brain, liver, breast, and thigh) in Ross 308 broilers for 42days. These authors observed that the deposition of different n-3 PUFA types in tissue was variable: the predominantn-3 PUFA in the liver and the brain tissue was docosahexaenoic acid (48 and 88%, respectively), whereas in the breast and the thigh tissue was ALA (65 and 86%, respectively). The accumulation efficiency of essential fatty acids (LA and ALA) in the breast and the thight issue, along with the deposition of n-3 PUFAs, showed a variable affinity of each fatty acid for the different tissue types (Nain *et al*., 2015).

In conclusion, the results of the present study indicate that feeding 80 g/kg camelina cake in broiler chicks, as a rich source of n-3 PUFA, had no negative impact on productivity, beneficially alter the plasma lipid status and fatty acids profile of lymphoid tissue.

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