Effects of dietary protein levels and protein-oleaginous sources on fatty acids composition of carcass fraction in broiler chickens

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SUMMARY

The present study was conducted to determine the effects of different dietary protein levels and two protein-oleaginous sources on carcass fatty acids (FA) composition in broilers at 42 d. Cobb 500 broiler chicks (n=1200) were randomly divided in 6 groups with 4 replications per treatment. Broilers were fed 6 different isocaloric diets in a 3 x 2 factorial design with 3 levels of protein [medium protein (MP), high (HP) and low (LP)] and 2 protein-oleaginous sources (camelina cake, CC and canola meal, CM). The proportion of CC and CM in broiler diets was 80 g/kg. The FA composition of carcass was determined by gas chromatography. The higher level of n-3 PUFA, especially ALA in CC compared to CM was reflected in carcass fraction n-3 PUFA composition, whatever the protein level. This effect is associated with the presence of long chain n-3 FA (eicosapentaenoic and docosahexaenoic) in carcass fraction. In addition, the use of CC decreased significantly n-6:n-3 PUFA ratio of carcass fraction (3.28:1 in CC diets vs. 6.65:1 in CM diets), with real benefits for human health. The chemical composition of carcass fraction was not influenced significantly by the protein level and sources. The dietary protein level had no significant effect on FA composition of carcass fraction. In conclusion, the results showed that LP diets can ensure similar carcass quality and FA profile than HP or MP diets. Our study highlight that the camelina cakes is more valuable dietary source of n-3 PUFA for broilers than canola meal.

Keywords**:** broilers carcass, n-3 fatty acids, protein, camelina cakes

INTRODUCTION

Manipulation of the dietary protein level has a major effect on performance and carcass composition of broiler chickens (Collin et al.

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2003). Decrease of the dietary crude protein (CP) lead to a decreasing in carcass protein and an increase in carcass fat content, due to the fact that protein has the ability to act as a lipotrophic agent (Si et al., 2001). Majority of the previous researches reported that decreasing CP content by more than 3% in broilers diets, reduced the performance and affects the carcass composition, even though all known essential amino acids (AA) requirements are assured (Sterling et al., 2005; Waldroup et al., 2005; Kamran et al., 2008; Namroud et al., 2008; Berres et al., 2010). Nowadays, carcass quality is of major important and interest in poultry industry due to increasing consumer interest in lean meat, low fat and production of product with added values (Zhan et al., 2007). Carcass fatty acids (FA) are key elements that control meat quality and have a primordial role in the nutritional feeding value of the meat (Wood et al., 2008). Dietary manipulation of the fatty acid profile could improve the concentrations of beneficial omega-3 polyunsaturated fatty acids (n-3 PUFA) in poultry meat (Rymer and Givens, 2005; Ribeiro et al., 2013). Thus, poultry meat has been considered an attractive way to increase n-3 PUFA in human diets (Rymer and Givens 2005; Poureslami et al., 2010; De Smet, 2012). Recently, the significant increase of by-products from biofuels industry (e.g. canola, flax, camelina meals/cakes) has attained interest for valorisation in poultry diets as vegetable sources rich in protein, energy and favourable FA composition. However, the findings on the effect of different dietary protein levels in isocaloric diets on carcass composition of broiler chickens are not elucidated, especially considering the utilization of different protein-oleaginous sources.

Therefore, the aim of study was to evaluate the effects of different dietary protein levels and protein-oleaginous sources [camelina cakes (CC) and canola meal (CM)] on carcass fatty acids composition in broilers at 42 d.

MATERIAL AND METHODS

The study protocol was approved by the Animal Care Committee of the National Research-Development Institute for Animal Biology and Nutrition (Balotesti, Romania) and birds were treated in accordance with Romanian law no. 305/2006 and EU Directive 2010/63/EU regarding handling and protection of animals used for scientific purposes (OJEU, 2010).

Broilers, diets and sampling

A total of 1200 broilers Cobb 500, 908.81 ± 59.07 g were randomly assigned into 6 dietary treatments, with 4 replicates per treatment (100 broilers/replicate). The broilers were subjected to a 1 phase feeding regimen consisting of finisher phase (23 to 42 d) and were fed with 6

different isocaloric diets (13.39 MJ/kg) in a 3 x 2 factorial design with 3 levels of protein: medium protein (M; 180 g/kg CP), high protein (HP; 200 g/kg CP) and low protein (LP; 160 g/kg CP) and 2 protein-oleaginous sources (CC and CM). The diets based on corn, wheat, soybean meal, corn gluten, synthetic amino acid (DL-methionine and L-lysine), were formulated to have similar content of sulphur amino acids, lysine, calcium and phosphorus (Cobb-Vantress, 2008). The inclusion level of CC and CM in broiler diets was 80 g/kg. The analysed compositions of finisher experimental diets are shown in Table 1.

Table 1. Nutrient composition of finisher experimental diets

	CC diets			CM diets		
Analysed composition[1] (g/kg)	MP	HP	LP	MP	HP	LP
Crude protein	181.0	200.7	162.0	181.6	199.9	160.8
Lysine, total	10.54	10.57	10.56	10.55	10.53	10.54
Methionine + cystine, total	8.16	8.18	8.19	8.18	8.19	8.20
Calcium	8.90	8.80	8.90	8.80	8.90	8.90
Phosphorus, total	8.30	8.40	8.40	8.20	8.30	8.30
Crude fibre	38.00	37.80	38.90	37.80	38.50	38.00
Ether extract	67.80	70.20	60.30	62.80	74.00	75.20
Metabolisable energy (MJ/kg)[2]	13.39	13.39	13.39	13.39	13.39	13.39

[1]Based on analysed chemical composition.
[2]Calculated using regression equations (NRC, 1994).

The broilers were housed in an environmentally controlled house and kept in pens on litter (wood shavings). Chicken vaccination was carried out according to the usual schedule. Lighting schedule was 23h light:1h darkness. Feed and water were provided *ad libitum.* Feed was withdrawn 12 h prior to slaughter.

At the end of the experimental period, 4 broilers from each replicate (2 males and 2 females) were randomly selected for carcass analyses. The broilers were successively weighed, killed by cervical dislocation, defeathered, dried and weighed again for calculation of the weight of feathers by difference. Afterwards, the oesophagus, trachea, proventriculus, gizzard, intestines, heart, liver, gall bladder, kidneys, lungs, spleen and Bursa of Fabricius were removed from the body. The remaining body, including the abdominal fat, formed the "carcass fraction". The carcass fraction was chemical analysed for FA profile estimation. The carcass fraction was weighed, grounded (TC-121 electric grinder, Maxigel, Italy) and homogenized. Samples of approximately 500 g were preserved at - 20°C until chemical analyses.

Chemical analyses

Standardized methods according to Commission Regulation (EC) no. 152/2009 (OJEU, 2009) were used to determine the gross chemical composition of protein-oleaginous sources, diets and carcasses samples.

Gas chromatography method was used to determine the fatty acid composition of carcasses samples. As previously reported Hăbeanu et al. (2015), the principle of the method consist in the transformation of the FA from the fat sample into methyl esters followed by the separation of the components in a capillary column and their identification by comparison with the standard chromatograms (reference standard purchased from the Romanian National Office for Standards). We used chromatograph with capillary column with high polarity stationary phase (BPX70, 60m × 0.25 mm inner diameter, 0.25 μm film); or high polarity cyanopropyl phases, which have similar resolutions for different geometric isomers (THERMO TR-Fame 120m × 0.25 mm ID × 0.25 μm film).

Statistical analysis

The data obtained were analysed using the GLM procedure of the software SPSS (IBM SPSS Statistics version 20.0, 2011). One-way analysis of variance (ANOVA) with the post hoc Tukey's multiple comparison test was used to evaluate statistical significance of differences between dietary treatments. The results are given as means and standard error of the mean (SEM). The values for fatty acids are expressed as percentage (% of total FAME). Differences were considered significant at $P \leq 0.05$. Replication was considered as the experimental unit for determined performance.

Table 2. Fatty acids profile of protein-oleaginous sources and finisher experimental diets (% of total FAME)[1]

Fatty acids	Camelina cakes	Canola meal	CC diets			CM diets		
			MP	HP	LP	MP	HP	LP
C14:0 (myristic)	0.15	0.38	0.10	0.10	0.10	0.09	0.10	0.10
C16:0 (palmitic)	7.43	12.04	8.77	8.51	8.96	8.76	9.06	9.29
C16:1 (palmitoleic)	0.24	2.42	0.24	0.24	0.23	0.21	0.23	0.24
C18:0 (stearic)	2.01	1.66	2.38	2.41	2.52	2.87	2.88	2.84
C18:1cis-9 (oleic)	17.69	44.80	19.92	19.25	20.06	24.80	24.67	24.98
C18:2n-6 (linoleic; LA)	21.09	31.60	45.37	46.05	44.62	61.31	60.96	60.65
C18:3n-3 (α-linolenic; ALA)	29.47	5.40	6.44	6.10	6.41	2.24	1.94	1.46
Total SFA	9.59	14.08	11.25	11.02	11.58	11.72	12.04	12.23
Total MUFA	17.93	47.22	20.16	19.49	20.29	25.01	24.90	25.22
LA:ALA ratio	0.72	5.85	7.04	7.54	6.96	27.37	28.48	27.84

[1]FAME: fatty acids methyl esters.

SFA: Saturated fatty acids; Total SFA = C14:0 + C16:0 + C18:0.

MUFA: Monounsaturated fatty acids; Total MUFA = C16:1 + C18:1cis-9.

RESULTS AND DISCUSSION

The fatty acids composition of protein-oleaginous sources and experimental diets are presented in Table 2. As expected, camelina cakes had the highest content in α-linolenic acid (ALA, 29.47% vs. 5.40% in canola meal). On the contrary, the linoleic FA (LA) was 49.83% lower in CC than CM. Therefore, the CC diets contained a high concentration (6.32%) of ALA and a ratio of 7.18:1 of LA:ALA compared to CM diets 1.88% ALA and 27. 89:1 of LA:ALA ratio, respectively.

As shown in Table 3, the dietary protein level or protein-oleaginous sources had no effect (P >0.05) on the chemical composition of broilers carcass at 42 d of age. As expected, the protein content of carcass fraction increase in HP and decrease in LP vs. MP, and inversely, the fat content decrease in HP and increase in LP vs. MP (P >0.05). The results of present study are in agreement with previous studies who reported that decrease in dietary CP level had no negative effect on carcass composition (Hai and Blaha, 2000; Si et al., 2001; Bregendahl et al., 2002; Kamran et al., 2008; Gous et al., 2012; Gheorghe et al., 2013). Conversely, Namroud et al. (2008) reported no significant difference in whole body protein content, but dietary protein level affected the whole body fat inversely. According Namroud et al. (2008), one of the mechanisms involved in decreasing carcass fatness ass effect of higher protein level in the diets is associated with increased heat increment involved in deamination and transamination of surplus amino acids to other metabolites and finally uric acid.

Table 3. Chemical composition of carcass fraction from broiler chickens fed with different dietary protein levels and protein-oleaginous sources[1] (%)

Item	Camelina cakes			Canola meal			SEM	*P*-value of effects[2]		
	MP	HP	LP	MP	HP	LP		PL	POS	PL×POS
Dry matter	31.79	33.34	30.82	33.84	33.35	31.83	0.642	0.78	0.16	0.57
Crude protein	17.06	17.48	16.81	17.11	17.80	16.95	0.218	0.64	0.33	0.29
Fat	10.98	10.90	11.33	12.17	10.61	12.96	0.119	0.07	0.24	0.98
Ash	3.09	2.78	3.11	3.26	2.85	3.19	0.078	0.16	0.10	0.21

[AB]Means within rows with different superscripts are significantly different at $P < 0.01$.
[ab]Means within rows with different superscripts are significantly different at $P < 0.05$.
[1]n =4 for each dietary treatments, pooled SEM (Standard error of means).
[2]PL: protein level; POS: protein-oleaginous sources; PL × POS: interaction between protein level and protein-oleaginous sources.

The effects of dietary protein level and protein-oleaginous sources on fatty acids profile of broilers carcass at 42 d of age are shown in Table 4.

Table 4. Fatty acids composition of carcass from broiler chickens fed with different dietary protein levels and protein-oleaginous sources* (% of total FAME)[1]

Item	Camelina cakes			Canola meal			SEM	*P*-value of effects[2]		
	MP	HP	LP	MP	HP	LP		PL	POS	PL×POS
16:0	29.16	31.28	29.18	27.98	30.56	28.96	0.380	0.14	0.47	0.92
C16:1	6.59^{A}	6.15^{A}	7.36^{A}	4.72^{B}	5.40^{B}	5.45^{B}	0.298	0.31	<0.002	0.45
C18:0	7.63	7.67	7.60	7.99	8.53	7.60	0.115	0.33	0.14	0.43
C18:1cis-9	37.05	39.25	37.82	37.82	37.90	38.53	0.303	0.32	0.94	0.28
C18:2n-6	9.03^{b}	7.25^{b}	8.22^{b}	13.28^{a}	11.40^{a}	11.51^{a}	0.993	0.22	0.03	0.97
C18:3n-3	0.98^{a}	0.73^{a}	0.90^{a}	0.34^{b}	0.17^{b}	0.25^{b}	0.144	0.17	0.02	0.34
CLA	0.03	0.08	0.15	0.37	0.25	0.20	0.421	0.50	0.10	0.06
C20:2n-6	1.53^{A}	2.18^{A}	1.71^{A}	0.47^{B}	0.55^{B}	0.66^{B}	0.510	0.39	<0.0001	0.44
C20:3n-3	0.91	1.16	0.90	0.84	1.14	1.01	0.025	0.28	0.65	0.69
C20:4n-6	0.10	0.08	0.05	0.19	0.16	0.10	0.108	0.12	0.56	0.74
C20:5n-3	0.69^{a}	0.63^{a}	0.56^{a}	0.44^{b}	0.42^{b}	0.39^{b}	0.118	0.15	0.04	<0.01
C22:6n-3	0.62^{a}	0.60^{a}	0.57^{a}	0.36^{b}	0.28^{b}	0.25^{b}	0.221	0.97	0.03	0.45
Total SFA	36.79	38.95	36.78	35.97	39.09	36.56	0.864	0.08	0.85	0.87
Total MUFA	43.64	45.40	45.18	42.54	43.30	43.98	0.284	0.25	0.21	0.77
Total PUFA	14.71	12.59	13.12	16.02	13.32	14.27	0.656	0.15	0.70	0.95
PUFA:SFA ratio	0.40	0.32	0.35	0.45	0.34	0.39	0.059	0.14	0.73	0.95
Total n-6 PUFA	10.69	9.52	10.13	14.31	12.36	12.47	0.297	0.21	0.38	0.98
Total n-3 PUFA	3.20^{a}	3.12^{a}	2.93^{a}	1.98^{b}	2.01^{b}	1.90^{b}	0.173	0.30	<0.03	0.18
n-6:n-3 ratio	3.34^{B}	3.05^{B}	3.46^{B}	7.23^{A}	6.15^{A}	6.56^{A}	0.381	0.74	<0.01	0.59

^{AB}Means within rows with different superscripts are significantly different at $P<0.01$.
^{ab}Means within rows with different superscripts are significantly different at $P<0.05$.
*n=4 for each dietary treatments, pooled SEM (Standard error of means).
[1]FAME: fatty acids methyl esters; SFA: Saturated fatty acids; Total SFA = C16:0 + C18:0.
MUFA: Monounsaturated fatty acids; Total MUFA = C16:1 + C18:1cis-9.
PUFA: Polyunsaturated fatty acids; Total PUFA = C18:2n-6 + C18:3n-3 + CLA + C20:2n-6 + C20:3n-3 + C20:4n-6 + C20:5n-3 + C22:6n-3.
[2]PL: protein level; POS: protein-oleaginous sources; PL × POS: interaction between protein level and protein-oleaginous sources

The FA profile of carcass was not affected (P >0.05) by the dietary protein levels, but was positively influenced by the use of CC as protein-oleaginous source. The main effect of protein-oleaginous source on carcass FA profile showed that the palmitoleic acid was highest 6.70% in CC vs. 5.19% in CM (P <0.002). Feeding CC diets led to a significant decreases in LA content, as predominant n-6 PUFA, (8.17% vs. 12.06%; P =0.03) and a significant increases in n-3 PUFA (3.08% vs. 1.96%; P =0.03), especially ALA (0.87% vs. 0.25%; P =0.02) in carcass vs. CM diets. This effect is associated with the presence of long chain n-3 PUFA [eicosapentaenoic (0,63% vs. 0.42%; P =0.04) and docosahexaenoic (0.60% vs. 0.30%; P =0.03)] in carcasses, compared with CM diets. Long-chain PUFA are mainly formed in the liver from LA and ALA by the action of desaturases and

elongases (Wood et al., 2008). These FAs compete in the metabolism of the same enzymes, therefore higher dietary intake of ALA resulted in the increase of those FA in muscle tissue as well as an increase in its derivates DHA and EPA. This has positive implications for a consumer's health, because humans have a low ability to convert ALA to DHA and EPA (Burdge, 2006). Also, these precursors of ALA play an important role in the control of cardiovascular diseases (Givens, 2009).

Moreover, the use of CC decreased significantly n-6:n-3 PUFA ratio of carcasses (3.28:1 in CC diets vs. 6.65:1 in CM diets; $P < 0.01$), with real benefits for human health (Simopoulus, 2008; Hăbeanu et al., 2014). The interaction between protein level x protein-oleaginous sources was significant for eicosapentaenoic acid ($P < 0.01$).

Our results suggest that addition of 80 g/kg camelina cakes in finisher broiler diets improve the content of n-3 PUFA, especially ALA in carcasses of broilers. This is consistent with previous studies (Betti et al., 2009; Aziza et al., 2010; Cherian, 2012; Gheorghe et al., 2014) showing that feeding broilers with camelina by products up to 10 % enhanced the n-3 PUFA content of meat.

Conclusions

The results of present study showed that 10% reduction of the protein level of finisher diets supplemented with essential amino acids, can ensure similar quality of carcasses in terms of chemical composition with the high or medium protein diets. Moreover, the results highlight that the camelina cake is more valuable dietary source compared to canola meal in terms of lipid quality of carcass.

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