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CHANGES IN FATTY ACIDS COMPOSITION OF *Longissimus dorsi* MUSCLE, BRAIN, LIVER AND HEARTAS EFFECT TO HEMPSEED ADDITION IN PIGS FEEDING

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Abstract

The hempseed is a low-cost ingredient rich in n-3 fatty acids (FA). This study aimed to assess the FA concentration in different pig tissuesin combination with the performance as effect of the dietary addition of hempseed. Twenty hybrid Topigs (38.64 kg) were assigned to a 30d trial into 2 groups: C group received a basal diet and HS group received the same basal diet with 5% hempseed Jubileu variety. The FA were determined by gas chromatography. The n-3 rich diet altered highly significantly PUFA concentration in Longissimus dorsi, liver, heart and brain, especially n-3 PUFA (P<0.0001) without modifying significantly the performances. The brain and liver had registered the highest concentration of long chain FA in the HS diet (5.26% in liver, 5.04% in brain compared to 1.61% in heart). Long-chain FA were not identified in the Longissimus dorsi, whatever the diet. By addition of hempseed the C18:3n3 was increased significantly in all tissues except the brain. As the PUFA increased the peroxidability index also increased, especially in the liver and brain. We can conclude that the hempseed has a valuable nutritive potential, especially in n-3 PUFA, which led to a positive alteration of beneficial FAs.

Key words*: hempseed, n-3 FA, pigs, PUFA, tissue.*

INTRODUCTION

Hemp (*Cannabis sativa* L.) botanically is a species of fruits with Delta-D-Tetrahydrocannabinol (THC) content conform to European law (EU Council regulation 1782/2003, OJEU 2003). In recent years, interest has grown in the medicinal uses of hemp due to its remarkably low toxicity (Vinod, 2011). Industrial hemp, with content of delta-9-tetrahydrocannabinoid (THC) < 0.02 was accepted to be cultivated in EU countries. THC, the main psychoactive component of hemp, is an appetite promoting agent which occurs naturally in the nervous and immune systems of animals (Vinod, 2011) and may be the cause to overeating and metabolic syndrome in normal animals (Hayatghaibi and Karimi, 2007). Because of the hempseed content in highly digestible protein, amino acids and essential fatty acids (EFA), it could be marketed as a valuable product for animal feeding and as a health-promoting (Callaway, 2004; Russo and Reggiani, 2013). Hempseeds contain about 30% oil, more than 80% being unsaturated FA (Russo and Reggiani, 2013) which improves immunity (Khan et al., 2010). The essential FA as linoleic FA occurs at about 55%, linolenic FA about 20%, gamma-linolenic FA ranges from 1 to 4% and stearidonic FA is present at about 0.5-2.0%. These FAs govern growth, vitality and the state of mind. However, little is known about their functioning in the body (Lynn, 1992). The ratio of n-6: n-3 is near the ideal value for an efficient metabolic conversion (Callaway and Pate, 2009). Recent considerations suggest an optimal n-6: n-3 ratio between 2: 1 and 3: 1 (Simopoulos et al., 2000). Classical diets including soya or sunflower can lead to too much n-6 FA. Generally, the hempseeds are used for cattle and poultry. The very low digestibility of the fibre component limit its use in pig feeds (Göhl, 1982). The *Jubileu* hemp variety was approved in Romania for the production of seed and oil since 2012 (900-

1,200 kg seed/ha) and new ways for its valorisation in animal feeding are experimented. It is a very early variety, resistant to low temperatures in spring and fall. The aim of this paper was to assess the effect of including 5% *Jubileu* hempseed in pig diets on performances and distribution of FAs to different tissues. The level of 5% hempseed in pigs feeding is recommended by EFSA (2011), although no scientific data is available for edible tissues of the pigs or performances.

MATERIALS AND METHODS

Animals and diets

The animals were supplied by NRDIABN (INCDBNA) Balotesti, Romania. All experimental procedures were approved by the Ethical Committee of NRDIABN Balotesti in agreement with the legislation for the protection of animals. The feeding trial was carried out for 30 days on 20 hybrids Topigs [♀ Large White x Hybride (Large White x Pietrain) ♂ × Talent, mainly Duroc]. Animals were allocated randomly into two homogenous groups (C diet which consists of the basal diet without hempseed incorporated and HS diet with addition of 5% hempseed in the diet). The experimental animals showed no signs or indications of diarrhoea or other clinical disorders. The initial average weight was 38.64 ± 0.99 Kg. The nutrient composition of the feed was in agreement with that specified in the guide of Topigs hybrid (Table 1).

All pigs were given isolipid and isonitrogenous diets. Both of the groups had similar level of essential amino acids (lysine and methionine + cystine). The animals were fed *ad libitum* twice daily. The leftovers and the feed intake were recorded daily. The *Jubileu* variety of the hempseed replaced 30% of the soybean meal in the diet of the HS group. The hempseed was ground and screened through 8 mm mash sieves and analysed chemically.

Sampling, measurements and analyses

The animals were weighed in the beginning and at the end of the experiment in order to determine their body weight and the average daily gain. Twelve hours before slaughtering, the access of the animals to feed was restricted. All pigs were stunned electrically, exsanguined and trimmed at an authorized slaughterhouse. Around 150 g samples of muscles *Longissimus dorsi* from the right half carcass of each animal, liver, heart and brain was collected, in order to determine the lipids and FA centesimal composition. The tissue samples have been transformed into a homogenous fine powder with IKA® A 11 basic mill, using liquid nitrogen.

Table 1. Diets feedstuff and nutrient composition

Ingredients (g/kg as feed basis)	C diet	HS diet
Maize	629.1	600.2
Rice flour	100.0	100.0
Hemp seed (var. *Jubileu*)	-	50.0
Soybean meal	150.0	120.0
Sunflower meal	80.0	90.0
DL-Methionine	0.1	0.1
L-Lysine	2.8	3.2
Calcium carbonate	16.5	17.0
Monocalcium phosphate	6.5	4.5
Salt	4.0	4.0
Premix choline	1.0	1.0
Vitamin-mineral premix	10.0	10.0
Nutrient analysed composition (g/kg feed)		
Dry matter	867.9	867.4
ME (kcal/kg)	3028	3067
Crude protein	160.7	158.5
Ether extract	37. 6	38.2
Lysine	9.7	9.7
Methionine + cystine	6.0	6.0
Calcium	9.0	9.0
Phosphorus	6.8	6.9

Chemical composition. The gross chemical composition of the ingredients, compound feed and tissue were determined by standardized methods as per Commission Regulation (EC) no. 152 (OJEU, 2009). The crude protein of the ingredients and of the tissue was determined using a semiautomatic classical Kjeldahl method which consists in the decomposition of the feed sample by heating with sulphuric acid, in the presence of catalysts, to reduce the organic nitrogen to ammonium ions that can be determined by distillation followed by titration. The fat was extracted using an improved version of the classical method by continuous extraction in solvent, followed by fat measurement with Soxhlet after solvent removal. The crude fibre was determined with a classical semiautomatic Fibertec-Tecator method.

Fatty acids. The fatty acids were determined by gas chromatography using a Perkin Elmer-Clarus 500 gas chromatograph (Massachusetts,

United States), fitted with Flame Ionization Detector (FID) and capillary separation column with high polar stationary phase Agilent J&WGC Columns, (United States), DB-23 dimensions 60m x 0.250 mm x 0.25 µm. After lipid extraction from the samples, the FAs were transformed into methyl esters by transmethylation. The components were separated in the capillary chromatograph column. The methylated FAs from the sample were separated according to chain length, to the level of unsaturation and to the geometry of the double bonds (method described by Hăbeanu et al., 2011). The mean values for FA composition were presented as g FA/100 g total FA ester methyl (%).

Peroxidation index. The peroxidation index (IP) was calculated by the equation reported by Hu et al. (1989).

IP = (% dienoic × 1) + (% trienoic × 2) + (% tetraenoic × 3) + (% pentaenoic × 4) + (% hexaenoic × 5).

Antioxidant activity. The antioxidant activity of the HS was determined by the DPPH method according to Arnous et al. (2001) with slight modifications, as previously reported Chedea et al. (2016).

Cannabinoid analysis. The cannabinoid content was determined by the colorimetric method, which consists of a colour scale, with grades from 1 to 10, corresponding to 0-1.0% THC content.

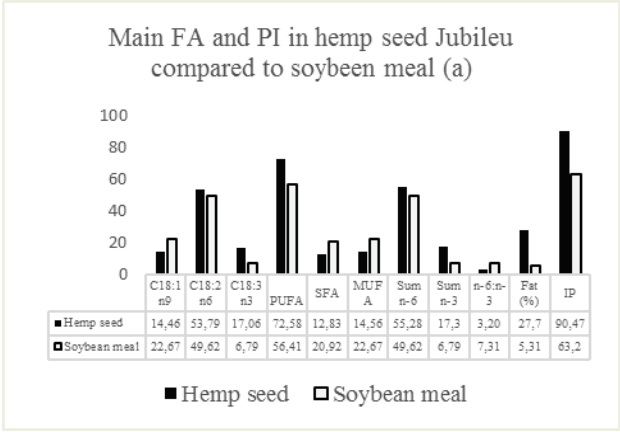
Statistical analysis

The data of the experiment were processed with SPSS - general linear model (SPSS, 2011) at 10%, 5%, 1% and 0.001% significance level. The response to the HS diet in different tissue was dependent variable, and the animal group was a fixed factor. The interaction of diet x tissue was determined as well. The results were expressed as mean value and standard error of the mean (SEM).

RESULTS AND DISCUSSIONS

Chemical composition

According to Regulation (EU) No 1307 (OJEU, 2013) areas used for the production of hemp are eligible hectares if the varieties have a THC content not exceeding 0.2%. Commission Regulation (EFSA, 2015) lists the feed materials that can be used in animal nutrition as hempseeds, hemp expeller and hemp oil. The hempseed used in our trial had the THC content of 0.0139%, lower than that noticed by EFSA (2015). In our study the hempseed *Jubileu* used had: 89.67% dry matter; 21.26% protein; 27.70 (%) fat; 28.82% cellulose. Figure 1 shows the FA composition of the hemp seed *Jubileu* compared to soybean meal (a) and the FA composition of diets (b).



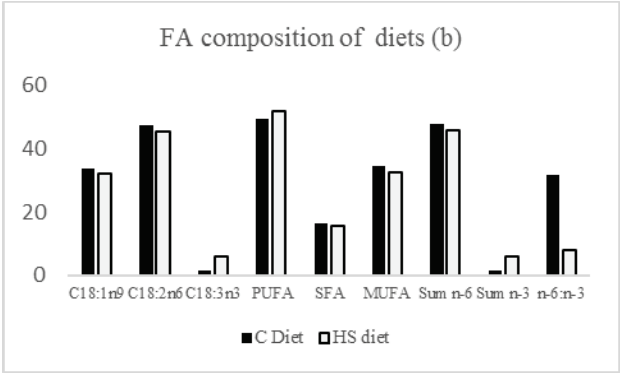


Figure 1. Fatty acids, fat and peroxidability index in hempseed var. *Jubileu*, soybean meal (a) and fatty acids composition of diets (b)

The concentration of C18: 3n-3 FA was close to that reported by Uluata and Zdemir (2012), 17.06% than 21.51%, respectively. The high concentration of n-3 and polyunsaturated FA (PUFA) of the hempseed *Jubileu* led to an n-6: n-3 ratio of 3.20. Positively correlated with this was the peroxidability index which was higher in hempseed than soybean meal (90.47 *vs.* 63.2). The soybean meal used in C diet had lower content in PUFA (56.41%) and of both n-6 (49.62%) and n-3 FAs (6.79%) compared to hempseed. The antiradical activity was 83.58 ± 2.36 µM TE (trolox equivalents)/g seeds.

Performance

EFSA (2011) noted that no data were available for pig feeding with hemp but suggested that 10

% hempseed cake or 5 % hempseed could be used for pigs. Our data confirm this hypothesis. Data on body weight (BW), average daily gain (ADG), feed intake (FI) and feed conversion ratio (FCR) are given in Table 2.

Table 2. Effect of the diets on zootechnical performance, carcass quality and lipid content of the tissue[1]

Performance	Dietary treatment		SEM	*P Value**
	C	HS		
Initial weight (kg)	39.10	38.30	0.24	*0.09*
Final weight (kg)	66.60	66.10	0.82	*0.78*
ADG (kg)	0.980	0.993	0.03	*0.83*
Feed intake	2.77	2.63	0.06	*0.30*
Feed: gain	2.85	2.65		
Carcass quality				
Back fat thickness (mm)	9.14	8.85	0.31	*0.66*
Muscle thickness (mm)	45.29	49.70	1.97	*0.28*
Lean meat (%)	60.89	61.76	0.44	*0.35*
Lipid content (g^{-1}DM)				
Longissimus dorsi	6.54	6.33	0.82	*0.91*
Liver	7.82	7.83	0.32	*0.99*
Brain	40.27	40.21	0.59	*0.96*
Heart	7.33	6.62	0.51	*0.52*

[1]N = 10 animals/treatment; C, control diet; HS, hempseed (var. *Jubileu*) diet; SEM, standard error of means.*P>0.05 no significate differences between groups.

Thus, an addition of 5% of hempseed in the diet had no adverse effects on pig performance. On the contrary, ADG was 6% higher in HS diet compared to C diet, whereas FCR was 12% lower than in C diet.

Table 2 also shows the results regarding the lipid content of *Longissimus dorsi* muscle, liver, brain and heart.

The lipid content of the tissue was not affected significantly by the diet. The brain had the highest level of fat, followed by the liver and heart.

Fatty acids transfer from diet to different organs

The changes of the fatty acids concentration as effect of two diets or tissue (*Longissimus dorsi,* brain, liver and heart) are presented in Table 3.

The dietary addition of hempseed as n-3 rich vegetable source is reflected in FA deposited in pig tissues.

The intake of C18: 3n-3 and PUFA was about 3.6, respectively 0.99 times higher in animals fed with HS diet, which increased C18: 3n-3 and PUFA level in the tissue and decreased n-6: n-3 ratio.

Table 3. Mean and SEM of fatty acids composition in the two diets distinguished by the level of n-3 PUFA and in *Longissimus dorsi* muscle, liver, brain and heart tissues as effect of the diets

Fatty acids (% of total FAME)	Diet		Tissue								SEM	*P value*	
			Longissimus dorsi		Liver		Brain		Heart				
	C	HS	C	HS	C	HS	C	HS	C	HS		*Diet*	*Tissue*
14:0	0.74	0.70	1.39	1.55	0.41	0.38	0.42	0.31	0.77	0.57	0.07	*NS*	***
16:0	19.23	18.21	25.15	25.31	16.28	13.98	16.72	16.41	18.76	17.12	0.66	*	***
16:1	1.96	1.70	3.33	3.26	1.06	0.87	1.64	1.40	1.84	1.28	0.15	+	***
18:0	17.63	18.02	12.73	12.09	22.72	23.53	20.93	21.95	14.12	14.52	0.75	*NS*	***
Total *trans* 18:1	0.10	0.10	-	-	0.32	0.30	0.06	-	-	0.10	0.02	*NS*	***
18:1*c*-9	27.34	25.56	44.46	42.97	15.42	13.29	25.39	25.32	24.06	20.64	1.76	*	***
18:2n-6	11.67	12.98	9.04	10.22	14.78	18.09	1.59	0.96	21.28	22.67	1.28	*	***
18:3n-3	0.30	0.96	0.24	1.09	0.34	0.91	0.25	0.13	0.37	0.91	0.15	*	*NS*
18:4n-3	0.28	0.33	0.06	0.01	0.25	0.22	0.76	0.91	0.05	0.17	0.05	*NS*	***
CLA	0.59	0.66	-	-	0.33	0.42	1.65	1.75	0.38	0.47	0.10	*NS*	***
20:3n-3	0.34	0.39	0.08	0.17	0.50	0.56	0.40	0.42	0.37	0.43	0.03	*	***
20:4n-6	8.30	8.41	1.26	1.20	16.52	15.61	8.52	9.08	6.91	7.75	0.86	*NS*	***
22:1n-9	0.07	0.05	0.02	0.01	0.01	-	0.05	0.05	0.20	0.15	0.01	*NS*	***
20:5n-3	0.23	0.43	-	-	0.62	1.22	0.12	-	0.18	0.48	0.06	***	***
22:5n-3	0.59	1.00	-	-	1.76	2.74	0.19	0.31	0.38	0.95	0.15	***	***
22-6n-3	1.32	1.56	-	-	0.89	1.30	4.34	4.73	0.02	0.18	0.30	*NS*	**
Total SFA	38.78	37.97	40.10	39.72	41.20	39.48	39.15	39.51	34.69	33.17	0.50	*NS*	***
Total MUFA	34.81	32.73	48.38	46.63	19.95	16.86	37.49	37.70	33.43	29.73	1.73	***	***
Total PUFA	25.77	28.59	11.49	13.43	38.25	42.84	22.55	22.34	30.81	35.85	1.78	***	***
Total n-6 PUFA	22.54	23.79	11.09	12.14	33.68	35.62	16.14	15.68	29.26	31.71	1.59	*NS*	***
Total n-3 PUFA	3.16	4.72	0.39	1.29	4.42	6.96	6.28	6.68	1.54	4.09	0.41	***	***
n-6:n-3	15.48	8.31	31.28	16.74	7.64	5.17	2.60	2.37	20.44	8.92	1.67	***	***
PI (%)[1]	53.72	59.98	14.76	17.44	85.18	93.99	67.34	69.29	47.61	59.36	4.43	***	***

SEM, standard error of means; NS (P>0.10); + (P<0.10); *P<0.05; **P< .01; ***P<0.001.

FAME, fatty acids ester methyl; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; Total SFA : C8:0+ C10:0+ C12:0+ C14:0 + C15:0 + C16:0 + C17:0 + C18:0+ C20:0+C24:0; total MUFA: C15:1+ C16:1 + 17:1 + Total *trans* 18:1+ C18:1cis-9 + C18:1 cis 7 + C20:1 n-9 + C22:1 n-9 + C24:1n-9; Total PUFA: C18:2n-6 + C18:3n-6 +C18:3n-3 + C18:4 n-3 + CLA + C20:2n-6 + C20:3n-3 + C20:4n-6 + C22:2n-6 + C20:5n-3 + C22:3n-6 +C22:3n-3+ C22:4 n-6 + C22:5 n-3 + C22:6n-3.[1]PI, peroxidability index.

Thus, highly significant effects of hempseed addition in the diet mainly concerned the unsaturated FA (the concentration of MUFAs were 1.06 times lower in HS diet and that of PUFAs were 1.06 times higher in HS diet), total n-3 FA (1.49 times higher in HS diet than C diet), C20: 5n-3 (1.86 times higher by addition of hempseed) and C22: 5n-3 FA (1.69 times higher in HS diet compared to C diet).

An pronounced effect had the dietary treatment to the following FA: C18: 3n-3 (P = 0.017), C18: 2n-6 (p = 0.025), C20: 3n-3 (P = 0.038), *cis*-9 -18: 1 (P = 0.019), C16: 0 (P = 0.047). Due to high dietary level of n-3 FA in HS diet the ratio n-6: n-3 decreased significantly (1.86 times). As we anticipated, while the level of the unsaturated FA increased, the SFA decreased. Correlated positively with the level of the PUFA, the PI was highly significantly influenced by the diet as well, due to their overall susceptibility to peroxidation.

The higher tissue incorporation of C18: 3n-3 with n-3 lipid supplemented diet is reflected in long chain FA, i.e. C22: 5n-3 FA (1.55 times higher deposition in the liver, 1.63 times higher deposition in the brain, 2.5 times higher level in heart tissue respectively) and C22-6n-3 (1.46 times higher in liver tissue, 1.08 times higher in the brain and 9 times higher in the heart). C20: 5n-3 FA was not identified in *Longissimus dorsi* and in the brain of HS diet animals as well, while in the liver the HS diet had the most pronounced effect (P<0.0001). The higher deposition of total n-3 FA in all tissues with HS diet (P<0.0001) highly stimulated the decrease of the n-6: n-3 ratio. Except the brain, total n-6 PUFA increased (P<0.0001).

The mean value of C20: 4n-6 arachidonic FA was influenced (P<0.0001) by the tissue. The lowest concentration of this FA was in *Longissimus dorsi* muscle whereas the highest level was in the liver of C group. The *cis*-9-18:1FA was influenced (P<0.001) by the tissue. Except the brain, the saturated FA (SFA) decreased (P<0.0001) in the tissue from HS group although the dietary treatment did not have significant effect. The liver had the higher concentration of total SFA in C diet. The most predominant SFAs were (P<0.0001) by the tissue. The interaction between the dietary treatment and tissue was highly significant for C20: 5n-3 and C22: 5n-3 long chain FA. In all tissues, the PI increased by addition of hempseed in the diet due to high level of PUFA more exposed to peroxidation processes.

DISCUSSIONS

The studies published on the physiological effects of the hempseed, especially on pig are few, even though hempseed, as n-3 FA and essential amino acids rich source, has been used for many years in human diets. As we showed above, the nutrient value of hempseed used in our study pointed an elevated concentration of n-3 PUFA, a ratio n-6: n-3 of 3.20 close to the level recommended for a positive therapeutic effect and stronger antioxidant properties reflected in the antiradical activity. In our study a 5% level of hempseed in the diet of the finishing pigs did not affect significantly the growth performances and carcass characteristics. Our results are in line with the study of Mourot and Guillevic (2015) and confirmed previous results obtained using other n-3 rich sources as camelina oil, camelina meal, linseed oil (Leskanich et al., 1997; Hăbeanu et al., 2011; Hăbeanu et al., 2014b) or by changing the n-3: n-6 ratio (Duan et al., 2014).

To our knowledge, except Mourout and Guillevic (2015) who tested the effect of hemp oil on fatty acids incorporation in *Longissimus dorsi* muscle, backfat and liver, there are no data reporting about the distribution of FA in different organs of the pigs as effect of dietary addition of the hemp. The present paper reported FAs variation between *Longissimus dorsi* muscle, liver, brain and heart. The *Longissimus dorsi* muscle, liver, heart and brain contained significantly different proportions of total lipid but there was no effect of the diet. It is known that n-3 FA metabolism is influenced by n-6 FA metabolism (Thomas et al., 2009) and both C18: 3n-3 and C18: 2n-6 are precursors of the long chain FA which has significant importance in the immune reaction and in brain development (Goyens et al., 2006; Sirot et al., 2008). However, there still is controversy on the capability of the dietary C18: 3n-3 to be converted into adequate levels of long chain FA, especially C22:6n-3 (Barcelo-Coblijnand Murphy, 2009). While the long chain FA (C20: 5n-3 and C22: 5n-3) were

increased by the dietary addition of hempseed ($P<0.0001$), C22: 6n-3 was not affected significantly by the diet. The probable explanation on this insignificant influence of the diet on C22: 6n-3 consists in the competition for Δ6 desaturase activity between C18: 3n-3 and the precursor for C22: 6n-3 (Cameron et al., 2000). Probably that the location of lipogenesis synthesis the tissue influenced differently the deposition of long chain FA. Previous studies are controversial regarding the effect of C18: 3n-3 rich diet on C22: 6n-3 at tissue level (Enser et al., 2000; Corino et al., 2008; Hăbeanu et al., 2014a).The most pronounced effect on the long chain FA was noticed in the liver and brain, whereas these FA were not identified in the *Longissimus dorsi* irrespective of the diet, contrary to the studies of Mourot and Guillevic (2015) with hempseed oil-based diet, or Hăbeanu et al. (2014b) with linseed oil in the diet. As anticipated, our data confirmed that the diet rich in n-3 FA by addition of hempseed markedly changed the level of the total n-3 PUFA ($P<0.0001$). The n-6: n-3 ratio was reduced highly significantly as well, in agreement with Mourot and Guillevic (2015) with hemp oil-supplemented diet, and Hăbeanu et al. (2014b) with linseed oil-based diet.

Generally, it is highlighted the fact that in pigs, the saturated FA are deposited in a higher concentration in tissues than the unsaturated FA, but using the n-3 PUFA rich diet, this statement changed. Thus, in our study the total SFAs decreased except the brain in all tissues of HS group especially due to the C16: 0 FA which was significantly affected by the diet. The distribution of C18: 3n-3 clearly appeared to be influenced by the diet than by the tissue, without any interaction between diet x tissue. Unexpectedly, in the brain the concentration of C18: 3n-3 was lower in HS diet than in C diet probably due to its higher conversion into total long chain FA (8% higher in HS diet than C diet). The liver and brain had the highest concentrations in n-3 PUFA and C20: 4n-6, which led to a higher value of the PI in these tissues. The accretion of C20: 4 n-6 at a high level in the brain is crucial for brain development. Although the diet had no significant influence on this FA, the tissue had a highly significant effect (1.82 times higher in the liver than in brain, 2.19 times higher than in heart and 13.05 times higher than in *Longissimus dorsi,* respectively).

CONCLUSIONS

A 5% level of hempseed incorporated in the diet markedly changed the PUFA concentration in *Longissimus dorsi* muscle, liver, heart and brain without altered the performances. The lipid contend was not affected by the dietary treatment. It seems that the brain and liver displayed the most pronounced effect of the n-3 rich diet on long chain FAs. The hempseed supplemented diet had a positive effect on n-3 PUFA and correlated negatively with this on n-6: n-3 ratio. However, the higher level of unsaturated FA increased the level of the peroxidability index. The liver is more susceptible to peroxidation than other tissues, follow by the brain that had a higher level of lipid on dry matter bases. Thus, in order to increase the level of incorporation of hempseed, the addition of antioxidant is advised.

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