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Performance and Fatty Acid Composition of Adipose Tissue, Breast and Thigh in Broilers Fed Flaxseed: A Review

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ABSTRACT

Interest on the enrichment of poultry meat with n-3 fatty acids has increased given its important role in human health. Flaxseed is the main sources of n-3 fatty acids, and contains between 45 to 71% of total fatty acids of the oil as α-linolenic acid. This paper reviews the plausibility of n-3 enrichment. Its focus is on the processing of flaxseed and begins by summarizing the benefits of supplementation on broiler performance. The literature on altering the FA deposition in different tissues is then reviewed, and the factors that affect the incorporation of n-3 PUFA into edible tissues of poultry are investigated. Flaxseed supplementation caused a reduction in the abdominal fat pad, and the main fatty acid deposited in the tissue is LNA. The use of fold-change analysis allowed interpreting and determining the variation of results within experiments that do not report data in similar units of measure. The fold change analysis identified three categories of desaturation response to feeding flaxseed to broilers, resulting in different values for EPA and DHA in both breast and thigh tissues: high, medium and low fold-changes. The use of flaxseed oil, whole or ground flaxseed 14 to 21 day before slaughter is recommended to poultry producers as feeding strategies to optimize n-3 enrichment, without compromising animal performance. Enriched DHA deposition could be accomplished feeding whole flaxseed.

Key words : Abdominal fat, Flaxseed, Intake, α-linolenic acid, n-3 enriched broiler meat.

INTRODUCTION

World-wide health professionals are emphasizing the need to increase intake of n-3 polyunsaturated fats, while reducing trans-fatty acids, saturated fatty acids and cholesterol due to its role in the prevention and treatment of coronary heart disease, major depression, aging and Crohn's disease, ulcerative colitis, and lupus erythematosus[1,2,3,4]. Omega-3 fatty acids, especially EPA and DHA, are important in mitigating platelet aggregation, blood triglycerides and cholesterol levels, as well as the occurrence of blood clots, and show both antithrombotic and anti-inflammatory effects[5]. On the other hand, DHA is a vital component in the retina and the membrane phospholipids of the brain[6]. Be that as it may, the most important n-3 fatty acids in human nutrition are eicosapentaenoic acid (EPA; 20:5n-3), docosahexaenoic acid (DHA; 22:6n-3), and α-linolenic acid (LNA; 18:3n-3), which serves as a precursor for EPA and DHA synthesis[7,8]. As people become more concerned about health issues, demand for more healthy chickens and eggs has risen. Nowadays, farmers are looking at the possibilities to include in the feed of chickens more flaxseed due to its nutritional properties.

Flaxseed or linseed (*Linum usitatissimum* L) contains 42 to 46% oil, of which 45 to 71% is α-linolenic acid (LNA; 18:3n-3)[9,10,11], it is also an excellent source of polyunsaturated fatty acids, moderate levels of monounsaturated fat, and low levels of saturated fat[12].

Flaxseed is known for having a number of anti-nutrients[13,14] including cyanogenic glycosides, mucilage and a vitamin B6 (Pyridoxine) antagonist called linatine. Apparently, the limiting consumption of flaxseed is due to the presence of these anti-nutrients.

Since flaxseed fat should be readily available for chicks, processing of flaxseed could be a very important step to maximize the potential of flaxseed as an alternative n-3 source for meat enrichment. Research spanning three decades suggests that feeding flaxseed in chickens would significantly alter the fatty acid composition and improve the n-3 content of poultry meat. It is the objective of this review, to synthesize, summarize and compare the impact of different feeding strategies and processing alternatives of flaxseed on performance and fatty acid deposition in edible tissues of poultry using fold change analysis.

Effects of Dietary Composition and Processing of Flaxseed on Performance of Broilers

It is known that the incorporation of flaxseed into livestock feed, especially for poultry, serve as source of energy, protein and α-linolenic acid. A number of studies have been conducted to determine how feeding flaxseed oil to broilers influences their performance. In general, flaxseed oil is readily accepted by broilers, and feeding up to 6% of the total dietary matter had no effect on dry matter intake and on body weight[15,17,16] and An *et al*., 1997[19]). This may be partially explained by the fact that digestibility of unsaturated fats is higher than saturated fats[18]. Interestingly, broilers fed 10% flaxseed oil from 28 d to 48 d or 53 d showed an improve or not different final body weight and weight gain when compared to a control diet, and feed efficiency was not affected by treatments[19,20].

In trying to reduce the high cost of the use of n-3 sources in commercial diets, to maximize the use of flaxseed, and to achieve desirable levels of n-3 fatty acids in meat, non-conventional feeding strategies have been used[21, 22,23]. Most of the experiments, analyzed here, carried out on the effect of feeding whole flaxseed have shown that inclusion levels of up to 20%, on a dry matter basis, adversely affected feed conversion efficiency, body weight and weight gain[24]. Broiler meat can be enriched if birds were fed differently, and they used a feeding system that incorporated whole flaxseed at 7 and 14 days prior to slaughter. In addition, optimal dietary intake of whole flaxseed was reported, where the addition of 10% flaxseed did not affect carcass and live weights of birds[21, 25].

Some studies have shown that feeding ground flaxseed at dietary levels in excess of 7.5% in the dry matter, reduced growth rate and body weight[26,23], resulting in a poor feed conversion efficiency[29,28].This could be explained by the fact that impaired performance of broilers is accompanied with drastic reduction in the protein efficiency ratio (PER) and net protein ratio (NPR)[26], mainly due to a reduction in the retention of nitrogen and amino acids caused by the presence of mucilage[29].

Pelleting is a conventional processing procedure that includes the use of pressure and heat. High pressure, increased friction and heat are factors that modify the seed physical structure and cause the lipid content to be more available to digestive enzymes in broilers. It is expected that poisoning by cyanogenic glycosides will be greatly diminished if the endogenous enzymes are inactivated by heat[30]. Chicks fed 10% of the total dry matter as pelleted flaxseed increased their body weight and feed consumption[31].Clearly, pelleting primarily improves energy and nitrogen efficiencies[32]. It increases the density of the feed and breaks down the cells of the grains, and wet heat can make the nutrients (other than starch) more available. Lately, it waspointed out that the use of NSP-degrading enzyme in a 15% whole flaxseed pelleted diet fed to young birds (18 d) did not affect BW gain[32].

The presence of cyanogenic glycosides in flaxseed has been the major problem with its utilization in poultry diets. Thus, heat treatment of flaxseed has been used to reduce these compounds[32]. Some studies were developed to test the effects of feeding heated flaxseed to broilers, and no deleterious effect on body weight and feed efficiency was achievable when broilers were fed heated flaxseed at 5%; however, the n-3 enrichment was not desirable or very low [34].

Usually young animals are more sensitive to the negative effect of increased intestinal viscosity; therefore, flaxseed meal inclusion should be avoided in young birds. Although, using water-boiled, water-soaked, flaxseed meal + flaxseed oil, autoclaved whole flaxseed or wet autoclaving flaxseed meal at 10% of the dry matter did not depress the growth of chicks[35,36,37,27].

Abdominal Fat Pad

Even though, the increase in carcass fat deposition of the modern broiler chicken continues to be a health concern of consumers, the waste product to the poultry processor and the added waste management problems. However, data obtained by some authors[40,15,19,39,20,39], showed that feeding flaxseed oil up to 10% of the dry matter to male and female broiler chicks, or up to 17% of whole flaxseed[24] reduced the abdominal fat pad. Low abdominal and body-fat deposition of broilers fed polyunsaturated fatty acids is considered to be a result of very high rates of lipid oxidation[40,41,42], accompanied with high lipogenic activity, reflected by the high ratio of SFA +n-7 +n-9[43,44].

Effect of Dietary Composition and Processing of Flaxseed on Composition and Fatty Acid Profile of Different Broiler Tissues

Earlier studies have demonstrated that feeding flaxseed-containing diets to broilers can modify the n-3 fatty acids content of meat. For this reason, many research studies have been concentrated to increasing the levels of these fatty acids in almost all products of animal origin. Even though, the increase in meat LNA is a gradual process[45]. Phetteplace and Watkins were the first to show that flaxseed increased n-3 fatty acids deposition in chickens. Tables 1, 2 and 3 summarize the fold change in abdominal, breast and thigh tissues PUFA analysis for a number of studies, whose objectives were to contrast the PUFA profile of broilers fed flaxseed supplemented diets and control diets. Regrettably, not all studies report data in similar units of measure (i.e., g/100 g of tissue), so direct comparisons between studies are not possible. Fold change is commonly used in microarray expression analysis; this is an intuitive and powerful method to quickly determine a change in the expression level of a gene[46,47], therefore, comparisons between studies can be done by using fold change analysis. This is the first time that such type of analysis is done to compare the impact of different feeding strategies and processing alternatives of flaxseed on fatty acid deposition in edible tissues of poultry.

Abdominal Fatty Acid Profile

Flaxseed oil is not typically employed in poultry diets as the preferred source of α-linolenic acid (LNA; 18:3n-3) due to its oxidative potential during storage and mixing feeds[48]. However, the effects of flaxseed oil supplementation on the abdominal fatty acid profile, in essence, showed that as the level of supplementation increased the fold change in fatty acid increased for LNA fatty acid (Table1). On the other hand, long-chain n-3 PUFAs were undetectable and total n-6 unchanged or decreased[16,49]. As noted in Table 1, LNA fold change varied from 7.96 to 27.17, those variations were due to the dietary fat sources used in the control diets. In astudy was compared flaxseed oil with a control diet based on tallow, which is a source of saturated fatty acids[39]. On the other hand, it was used sunflower oil as a control diet, which is considered a rich source of n-6 and n-9 fatty acids[15].

In a study with ground flaxseed LNA and total n-3 fatty acids were increased, while eicosapentaenoic (EPA), docosahexaenoic (DHA) and other derivatives of n-3 acids were not found[28]. The absence of long-chain n-3 PUFA could be the result of the fact that long-chain n-3 PUFAs are deposited in muscle fat rather than in abdominal fat. PUFAs are preferentially incorporated into phospholipids which are in higher proportion in muscle fat than in adipose tissue fat[50].

Breast Muscle Fatty Acid Profile

The main interest in feeding flaxseed to broilers in recent years is to increase n-3 PUFAs deposition in breast meat, since breast is the most popular poultry meat. Flaxseed fed broilers consistently produce high concentrations of PUFAs, which include FAs such as LNA, the primary PUFA in broilers.

Diet supplementation with flaxseed oil has had a generally high effect on LNA and LCn-3 fatty acids deposition during different stages of the broiler's life (Table 2). However, as shown in Table 2, LNA fold change varied from 1.83 to 50.33 in trials using flaxseed oil, showing an incredible high ratio in different experiments, which can be explained by the dietary fat sources used in the control diets of those experiments and the presence or not of skin. Even though, LNA fold-change could be lower than those found in some of the studies due to the fact that some authors did not report on whether the samples were analyzed with or without skin. According to some authors [21,52] the presence of skin dramatically increased n-3 content of all tissues analyzed, and specifically, LNA concentrations. Because, it is known that LNA is associated more with the triacylglycerols (TAG) rather than phospholipids[53], it is the skin that has the highest lipid content and the greater proportion of TAG, in which LNA accumulates[54]. On the other hand, LNA and LCn-3 fold changes varied by time of feeding with increased deposition occurring over time[49,39]. Negative fold change values were found in some studies, indicating that the control diets produced higher values of fatty acids than the tested diets[45, 55]. For example, in a study was used fish oil as a control diet, rich in LCn-3. Feeding 8.2% of flaxseed oil for 14 d before slaughter showed that under commercial settings n-3 fatty acids can be increased in meat, minimizing the cost of enrichment[45].

Humans have very limited capacity of conversion of LNA to EPA and DHA[56]. There is a theory that poultry has a relatively low capability to transfer LNA to EPA and DHA[57, 58]. Despite that theory, comparing the results of feeding strategies, the fold change analysis showed that the source of LNA, and feeding time affected the capacity of LNA to desaturate and elongate.

As illustrated in Table 2, fold change analysis identified three categories of desaturation response to feeding flaxseed: high, medium and low fold-changes. High fold-change of DHA with a value of 9.66 was determined when analyzing the results of [21]. In their study, broilers were fed 10% whole flaxseed 14 days before slaughter. Medium DHA ratio (6.62) resulted in the study of[59], when broilers were fed 2% flaxseed oil during 21 days; and low DHA ratio (<2.0) from all other studies reported in this review. Similarly, fold-change values for EPA were high (>28.0), medium (12.0) and low (>1.0). Even though, there is no evident linear relationship between the DHA concentration in the diet and the corresponding DHA content of tissue[54].

Table 1. Fold change in abdominal broiler fat of mean polyunsaturated fatty acid composition between flaxseed supplemented diets and control diets

Treatments	% Flaxseed DM	Days on diet (from - to)	A fold change in fatty acid (Ratio)						
			LNA[1]	EPA[2]	DPA[3]	DHA[4]	Total	Total (n-3)	Reference (n-6)
Flaxseed Oil	10	21 to 42	27.17	0.00	NR[5]	0.00	27.17	1.45	39
Flaxseed Oil	5	1 to 56	24.51	0.04	0.02	ND[6]	24.57	1.12	49
Flaxseed Oil	5	1 to 49	7.96	2.08	NR	NR	5.21	-1.22	15
Flaxseed Oil	4	11 to 42	13.53	0.00	0.00	0.00	13.53	-1.11	16
Ground Flaxseed	15	22 to 46	14.35	NR	NR	NR	14.35	NR	28

[1]LNA – α-Linolenic Acid [2]EPA – Eicosapentaenoic Acid[3]DPA – Docosapentaenoic Acid, [4]DHA – Docosahexaenoic Acid[5]NR – Not Reported[6]ND – Not Detected

Table 2: Fold change in breast muscle of mean polyunsaturated fatty acid composition between flaxseed supplemented diets and control diets

Treatments	% Flaxseed DM	Days on diet (from - to)	Skinless /Skin	A fold change in fatty acid (Ratio)						Reference
				LNA[1]	EPA[2]	DPA[3]	DHA[4]	Total (n-3)	Total (n-6)	
Flaxseed Oil	10	21 to 42	NR[5]	25.33	3.26	NR	1.81	9.26	-1.04	39
Flaxseed Oil	5	1 to 56	NR	32.35	>1.79	4.10	1.05	12.36	-1.19	49
Flaxseed Oil	2	1 to 21	NR	9.60	12.44	9.48	6.62	9.67	1.15	59
Flaxseed Oil	8.2	7 BS[6]	Skinless	2.65	-1.26	-1.12	-1.20	1.11	1.20	45
	8.2	14 BS		4.33	-2.31	-1.46	-2.35	1.07	1.33	
	8.2	35 BS		5.87	-5.18	-2.10	-9.04	1.09	1.52	
Flaxseed Oil	4	11 to 42	NR	16.66	2.7	1.72	-1.3	2.69	-1.09	16
Flaxseed Oil	1	25 to 40	Skinless	3.04	2.21	1.52	-1.04	NR	NR	55
Atalante	3			9.17	5.39	2.53	1.56	NR	NR	
	5			7.22	5.43	3.41	1.54	NR	NR	
	7			9.95	9.6	3.55	2.01	NR	NR	
Flaxseed Oil	8	1 to 50	NR	33.50	>1.82	9.17	2.02	NR	NR	61
Flaxseed Heated Oil	8			11.46	>1.63	10.00	2.26	NR	NR	
Flaxseed Heated Oil + á-Tocopheryl	8			50.33	>1.49	36.22	1.56	NR	NR	
Flaxseed Heated Ground	10	1 to 42	NR	3.00	1.42	1.16	-1.12	1.20	1.01	25
Flaxseed Meal + Canola Oil	6.5+3.5			4.09	1.61	1.14	-1.38	1.39	1.00	
Flaxseed Meal + Animal Tallow	6+4			2.10	-1.00	-1.18	-1.20	-1.06	-1.07	
Flaxseed Meal	5	1 to 42	NR	2.40	NR	NR	NR	NR	NR	62

Table 2 Continue

	10			4.72	NR	NR	NR	NR	NR	
	15			6.06	NR	NR	NR	NR	NR	
Pelleted Flaxseed	12	1 to 36	Skinless	2.77	3.13	NR	1.84	2.66	-1.36	52
			Skin	3.99	3.67	NR	1.09	3.81	-1.27	
Pelleted Flaxseed	12/15 PM	1 to 40	Skinless	4.23	1.47	1.35	-1.15	NR	NR	24
	14/17 PM			4.47	1.47	2.50	-1.50	NR	NR	
Whole Flaxseed	12/15 Whole			1.29	1.23	1.78	-1.50	NR	NR	
Whole Flaxseed	14 Whole /15 Mash			2.40	1.41	1.21	1.86	NR	NR	
Whole Flaxseed	10	1 to 42	NR	3.58	1.27	1.26	-1.25	1.19	-1.05	25
Whole Flaxseed	10	7 BS6	Skinless	3.27	>28.00	2.00	5.50	4.21	NR	21
	10	14 BS	Skinless	4.18	>43.00	3.72	9.66	6.71	NR	
	10	7 BS	Skin	2.58	>52.00	3.27	8.33	3.20	NR	
	10	14 BS	Skin	4.56	>90.00	6.09	14.83	5.68	NR	
Ground Flaxseed	15	22 to 46	Skinless	3.84	6.07	2.35	1.85	3.20	-1.22	28
Ground Flaxseed	10	4 BS	Skinless	1.16	-1.03	-1.06	-1.09	1.11	NR	22, 23
		8 BS		1.14	-1.16	1.00	1.26	1.12	NR	
		12 BS		1.44	1.19	1.02	-1.11	1.35	NR	
		16 BS		2.14	1.13	-1.06	-1.41	1.87	NR	
		20 BS		1.89	1.58	1.15	-1.09	1.73	NR	
	17	4 BS		1.78	1.39	1.20	1.31	1.66	NR	
		8 BS		2.49	1.96	1.30	-1.32	2.19	NR	
		12 BS		2.88	2.78	1.38	-1.10	2.54	NR	
		16 BS		3.11	2.41	1.48	-1.09	2.71	NR	
		20 BS		3.54	2.41	1.58	1.09	3.07	NR	

[1]LNA – α-Linolenic Acid [2]EPA – Eicosapentaenoic Acid[3]DPA – Docosapentaenoic Acid [4]DHA – Docosahexaenoic Acid[5]NR – Not Reported
[6]BS – Before Slaughter

Table 3: Fold change in thigh muscle of mean polyunsaturated fatty acid composition between flaxseed supplemented diets and control diets

Treatments	% Flaxseed DM	Days on diet (from - to)	Skinless /Skin	A fold change in fatty acid (Ratio)						Reference
				LNA[1]	EPA[2]	DPA[3]	DHA[4]	Total (n-3)	Total (n-6)	
Flaxseed Oil	10	21 to 42	NR[5]	29.72	2.97	NR	1.65	12.26	1.03	39
Flaxseed Oil	5	1 to 56	NR	29.50	>1.24	3.68	2.60	17.62	-1.13	49
Flaxseed Oil	2	1 to 21	NR	10.61	7.20	4.11	2.50	7.54	1.04	59
Flaxseed Oil	8.2	7 BS[6]	Skinless	4.42	-1.86	-1.86	-2.30	-1.12	1.10	45
	8.2	14 BS		8.06	-4.76	-3.92	-6.65	-1.03	1.29	
	8.2	35 BS		11.64	-15.17	-5.37	-18.97	1.21	1.49	
Flaxseed Oil Atalante	1	25 to 40	Skinless	3.02	2.89	2.09	1.01	NR	NR	55
	3			6.47	3.92	3.10	1.19	NR	NR	
	5			7.66	6.85	3.12	1.11	NR	NR	
	7			8.72	6.27	3.43	1.32	NR	NR	
Flaxseed Oil	8	1 to 50	NR	42.05	>0.81	5.21	1.91	NR	NR	61
Flaxseed Heated Oil	8			24.73	>0.99	23.50	2.97	NR	NR	
Flaxseed Heated Oil + α-Tocopheryl	8			34.41	>0.82	24.80	3.31	NR	NR	
Pelleted Flaxseed	12/15 PM	1 to 40	Skinless	4.61	>1.70	2.10	>0.20	NR	NR	24
	14/17 PM			5.02	>1.70	2.30	>0.30	NR	NR	
Pelleted Flaxseed	12	1 to 36	Skin	4.00	3.64	NR	1.48	3.82	-1.38	52
Flaxseed Heated Ground	10			4.18	1.60	1.23	1.03	1.61	-1.02	25
Flaxseed Meal + Oil	6+4			4.76	1.75	1.13	-1.13	1.98	-1.05	

Table 3 Continue

Flaxseed Meal + Tallow	6+4			2.08	1.00	-1.10	-1.12	1.06	-1.09	
Whole Flaxseed	10	1 to 42	Skinless	4.56	1.43	1.39	-1.01	1.63	-1.01	
Whole Flaxseed	12/15 Whole			1.59	>1.20	1.80	>0.30	NR	NR	24
	14 Whole/ 15 Mash			3.00	>1.50	2.10	>0.90	NR	NR	
Whole Heated	5	1 to 42	NR	-2.33	-5.00	1.25	-1.83	NR	NR	34
Flaxseed	10			1.91	1.10	2.37	1.72	NR	NR	
	15			2.83	2.60	3.25	3.09	NR	NR	
Whole Flaxseed	5			-3.25	-2.00	-2.00	1.37	NR	NR	
	10			-1.46	-1.25	-1.14	1.12	NR	NR	
	15			-1.38	-1.25	-4.00	-1.33	NR	NR	
Whole Flaxseed	10	7 BS	Skinless	2.90	>13.00	>13.00	>12.00	3.79	NR	21
	10	14 BS	Skinless	5.05	>35.00	>26.00	>34.00	7.26	NR	
	10	7 BS	Skin	2.61	>50.00	>33.00	>37.00	3.15	NR	
	10	14 BS	Skin	4.71	>105.00	>65.00	>80.00	5.82	NR	
Whole Flaxseed	5			1.69	-1.28	2.00	2.00	1.56	1.08	57
	7.5			2.00	-1.28	3.00	2.00	1.76	1.11	
Ground Flaxseed	5	8 to 42	Skinless	4.94	-1.50	3.00	3.00	3.64	1.12	
	7.5			6.87	-1.50	5.00	3.00	4.96	1.21	
Ground Flaxseed	15	22 to 46	Skinless	3.14	5.00	2.08	1.81	2.90	-1.26	28
Ground Flaxseed	10	4 BS	NR	1.40	1.28	NR	-1.76	1.31	-1.04	23
		8 BS		2.15	1.71	NR	-1.35	1.99	1.10	
		12 BS		2.23	2.02	NR	-1.33	2.07	-1.00	
		16 BS		2.35	2.33	NR	-1.64	2.19	-1.11	
		20 BS		2.78	2.27	NR	-1.24	2.56	1.00	

[1]LNA – α-Linolenic Acid [2]EPA – Eicosapentaenoic Acid[3]DPA – Docosapentaenoic Acid [4]DHA – Docosahexaenoic Acid[5]NR – Not Reported[6]BS – Before Slaughter

These results reflect the effect of feeding strategies on the elongation and desaturation of LNA and are in agreement with previous works. On the other hand, it was reported that dietary modifications could increase DHA deposition in broiler tissues[60].

In general, fold change analysis was able to determine variations between treatments in different studies. For example, in a study with broilers fed heated flaxseed oil supplemented with α-tocopheryl, the LNA fold-change was 50.33-fold increase compared to the control; EPA, DPA and DHA also increased >1.49 (control diet was reported zero content), 36.22 and 1.56-fold, respectively[61]. Negative ratio was found for DHA when heated ground flaxseed was compared with heated ground canola, rich in oleic acid[60].

LNA fold-changes varied from 2.40 to 6.06 in the studies that used flaxseed meal for broilers, showing that increased dietary flaxseed levels resulted in LNA increases[62].Similarly, inclusion of flaxseed in the diet of broilers as pelleted or pelleted-then mashed caused broilers to produce breast muscle with increased LNA fatty acid[24], and EPA at the expense of decreased concentrations of total (n-6) fatty acids[52].

Feeding whole flaxseed during the life cycle of broilers, at 7 and 14 days before slaughter resulted in LNA fold changes that varied from 1.29 to 5.80. EPA values were higher when the control diet was zero, and high fold-change values were obtained for DHA. The DHA values varied from -1.25 to 14.83, indicating high values for DHA deposition in those studies. Even though, when samples were analyzed without skin, the values were lower.

The fold change analysis of the experiments carried out on the effect of feeding ground flaxseed to broilers resulted in values that varied from 1.07 to 7.25. Once consumed, the body converted LNA to EPA, DPA and DHA, albeit at low efficiency. DHA fold-change values varied from 1.0 to -1.41.

Thigh Muscle Fatty Acid Profile

It was reported in rats a higher concentration of n-6 and n-3 PUFA in type I and type IIa fibers than type IIb fibers[63]. Nonetheless, the results from the fold change analysis showed that in breast of broilers the total n-3 fatty acids varied from 1 to 12.36, and from 1 to 17.62 in thigh, but the total n-6 fatty acids was not much altered in those tissues, and the fold change was not higher than 1.52. These observations are consistent and explain why a reduction in total n-6 was observed[64].

Several studies have been conducted, to evaluate the fatty acid composition of thigh muscle after feeding flaxseed oil (Table 3). The fold change analysis showed that the LNA ratio was between 3.02 and 29.71, EPA from 1.10 to 31, DPA from 1.42 to 15 and DHA from 1.01 to 3.25, demonstrating that elongation and desaturation is feasible in this tissue. Again, LNA fold changes varied by time of feeding with increased deposition occurring over time[45], and also by increasing the dosage of treatment[55].

Results (Table 3) showed that DHA deposition in thigh muscle followed the same trend found in breast tissue. The fold change analysis identified three categories of desaturation response to feeding flaxseed to broilers: high, medium and low fold changes. High fold change of DHA with a value of >12.0 was found in the study of[21], medium DHA ratio (>2.0) in the work of[59], and low DHA ratio (<2.0) in all other studies analyzed here. On the other hand, the fold-change determined for EPA was high (>13), medium (7.20) and low (>1.0). One important aspect in this study is the discover of the three categories of fold-changes in EPA and DHA in breast and thigh muscles, which represent a step forward in the possibilities of EPA and DHA enrichment. In this context, the US DHA offered the most protection against Alzheimer Disease in humans[65]. Also, LNA, EPA and DHA block the action of some compounds that cause inflammation and help the body's cells to work properly and be elastic (Caughey *et al*., 1996[66]).

The response of feeding whole and/or heated whole flaxseed resulted in fold changes in between 1.27 to 8.58 for LNA. The higher value was found throughout the work of[60]. On the other hand, the fold change analysis resulted in high values for DHA (>80) in the research of[21]; in both studies, samples were analyzed with skin, which explains the obtained values. Similarly, the fold change analysis in diets using ground flaxseed varied from 1.40 to 6.87 for LNA, 1.28 to 5.0 for EPA, -1.25 to 3.0 for DHA, and the response observed in those studies varied by time of feeding and by increasing the dosage of treatment. In this tissue, flaxseed triggered increases in LC-n3.

Overall, this review, from animal performance to fatty acid deposition using fold change, may represent a very important step in the knowledge of poultry production providing new insights about feeding strategies using flaxseed, enabling n-3 enrichment without compromising animal performance.

CONCLUSION

Wide promotion of n-3 fatty acids spanned a research campaign on the use of flaxseed in animal production, while emphasizing the need to increase intake of n-3 PUFA. The knowledge achieved so far on enriching poultry meat with n-3 PUFA has made possible the availability of enriched poultry meat. As illustrated in this review, fold change analysis was a good method to interpret and determine the variation of results within experiments that do not report data in similar units of measure. The fold change analysis identified three categories of desaturation response to feeding flaxseed to broilers, resulting in different values for EPA and DHA in both breast and thigh tissues: high, medium and low fold-changes. Flaxseed contains some anti-nutritional factors that can be avoided by the use of some feeding strategies to enrich chicken meat without compromising live performance. Increasing the concentration of n-3 PUFA in poultry diets results in an increase in the n-3 PUFA content of poultry meat, and the use of skin or skinless samples in the analysis of fatty acid composition had an effect on the FA deposited. Nutritionally meaningful amounts of LNA and LCn-3 have been achieved in breast and thigh muscles by manipulating feeding time, flaxseed concentration in the diets, and using a proper processing method. Three feeding strategies and processing alternatives of flaxseed were identified to optimize enrichment: The use of flaxseed oil, whole or ground flaxseed 14 to 21 days before slaughter can be recommended to producers. Excellent results in DHA deposition could be accomplished feeding whole flaxseed.

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