EFFECT OF DIETARY N-6/N-3 RATIO ON FATTY ACID DISTRIBUTION IN DIFFERENT RABBIT TISSUES

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ABSTRACT

Western human diet is characterized by an imbalanced intake of polyunsaturated fatty acids (PUFA) in term of low intake of n-3 and n-3 LCP (Long Chain PUFA). The n-3 PUFA have a lot of physiological functions; therefore, it is important to know the extent of their synthesis starting from α-linolenic acid (ALA) in various tissues, then the amount need to reach an adequate level in the organism. In this study, we investigated the fatty acids distribution in different tissues of rabbits fed diets with different n-3 intake and n-6/n-3 ratio. Fifteen New Zealand White male rabbits were divided into three experimental groups and fed respectively, control, flaxseed (10%) and fish oil (3.5%) diets. At the end of the trial, rabbits were sacrificed and brain, liver, testes, epididymis and prostates were collected for the analysis of fatty acids profile. Results demonstrated that administration of n-3 PUFA affected the fatty acid distribution of these tissues, especially the brain. Benefits of n-3 PUFA on physiological function are well known, so further studies are necessary to understand the distribution of n-3 PUFA/n-6 and how to integrate these in diets to achieve a desiderable level in tissues.

Key words: tissues, polyunsaturated fatty acids, flaxseed, fish oil.

INTRODUCTION

Western diets are characterized by an imbalanced intake of polyunsaturated fatty acids (PUFA). Different studies demonstrated the n-3 and n-6 fatty acid antagonism. High intake of linoleic acid (LA) leads to an increase in arachidonic acid (AA) in the body tissues and a subsequent decrease in n-3 content. Accordingly, high ratios of n-6 and n-3 fatty acids alter the fatty acid composition of different tissue in rodents (Bourre et. al, 1993), pigs (Blank et al., 2002) and humans (Clark et al., 1992) including alteration of the nervous system (Sanders et al., 1984). The ability of mammals to metabolize α-linolenic acid (ALA) into longer chain PUFA (≥ 20 C; LCP) is important for assuring optimal health. In fact, n-3 fatty acids affect a lot of physiological functions; therefore, it is important to understand the extent of their metabolism in the body tissues, then the amount that must be consumed to reach an adequate level of these in the organism.
For this purpose, in the present study we investigated the fatty acids distribution of the main functional (brain, liver) and reproductive (testes, epididymis, prostate) tissues of rabbit bucks fed diets with different n-3 sources.

MATERIALS AND METHODS

Animals and experimental design

Fifteen New Zealand White male rabbits of 170 days of age were selected and divided into three experimental groups (n= 5/group) and fed different diets (**Table 1**):

• CONTROL group;
• FLAX group was fed with the control diet added with 10 % of extruded flaxseed;
• FISH OIL group was fed with the control diet containing 3.5 % of fish oil (NORDIC 117NATURALS omega-3®).

Table 1. Formulation and proximate analysis of the control and n-3 enriched diets

Ingredients		Control	Flaxseed	Fish oil
Dehydrated alfa alfa meal	g/kg	300	380	380
Soybean meal 44%	"	150	100	150
Barley meal	"	410	310	335
Wheat bran	"	52	52	52
Soybean oil	"	30	–	–
Extruded flaxseed	"	–	100	–
Fish oil*	"	–	–	35
Beet molasses	"	20	20	10
Calcium carbonate	"	7	7	7
Calcium diphosphate	"	13.5	13.5	13.5
Salt	"	7	7	7
DL-methionine	"	0.5	0.5	0.5
Vitamin-mineral premix	"	10	10	10
Crude protein	g/kg	175	174	175
Ether extract	"	48	47	42
Crude fiber	"	124	137	130
Ash	"	89	84	90

* NORDIC NATURALS omega-3® = purified deep sea fish oil (from anchovies and sardines) containing EPA 330 mg/100 g, DHA 220 mg/100 g, other n-3 LC PUFA 140 mg/100 g + α-tocopherol for preservation. Per kg diet: vitamin A 11.000 IU; vitamin D3 2000 IU; vitamin B1 2.5 mg; vitamin B2 4 mg; vitamin B6 1.25 mg; vitamin B12 0.01 mg; alpha-tocopheryl acetate 200 mg; biotine 0.06 mg; vitamin K 2.5 mg; niacin 15 mg; folic acid 0.30 mg; D-pantothenic acid 10 mg; choline 600 mg; Mn 60 mg; Fe 50 mg; Zn 15 mg; I 0.5 mg; Co 0.5 mg.

At the end of the trial (110 days) rabbits were sacrificed after stunning and brain, liver, testes, epididymis, prostate were accurately removed, sampled in sterile tubes and stored at -80 °C for the evaluation of fatty acid profile.

Chemical Analyses

The lipid extraction from tissues was performed according to Folch et al. (1997) and the esterification performed according to Christie (1982). The trans-methylation procedure was conducted using enicosenoic acid methyl esters (Sigma Chemical Co.) as internal standard. Fatty acids composition was determined using a Varian gas-chromatograph (CP-3800) equipped with a flame ionization detector and a capillary column of 100 m length × 0.25 mm × 0.2 μm film (Supelco, Bellefonte, PA, USA). Helium was used as the carrier gas with a flow of 0.6 mL/min. The split ratio was 1:80. The oven temperature was programmed as reported by Mattioli et al. (2019). Individual FAME were identified by comparing the relative retention times of peaks in the sample with those of standard mixture (FAME Mix Supelco; 4: 0 to 24: 0) plus cis-9, cis-12 C18:2; cis-9 cis-12 cis-15 C18:3; cis-9 cis-12 cis-15 C18:3 (all from Sigma Aldrich). Fatty acids were expressed as % of total FA. The average amount of each fatty acid was used to calculate the sum of the total saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and PUFA. To evaluate the efficiency of metabolizing precursors (LA and ALA) into LCP the ratio LCP/precursors were calculated for both n-3 and n-6 (Castellini et al., 2016).

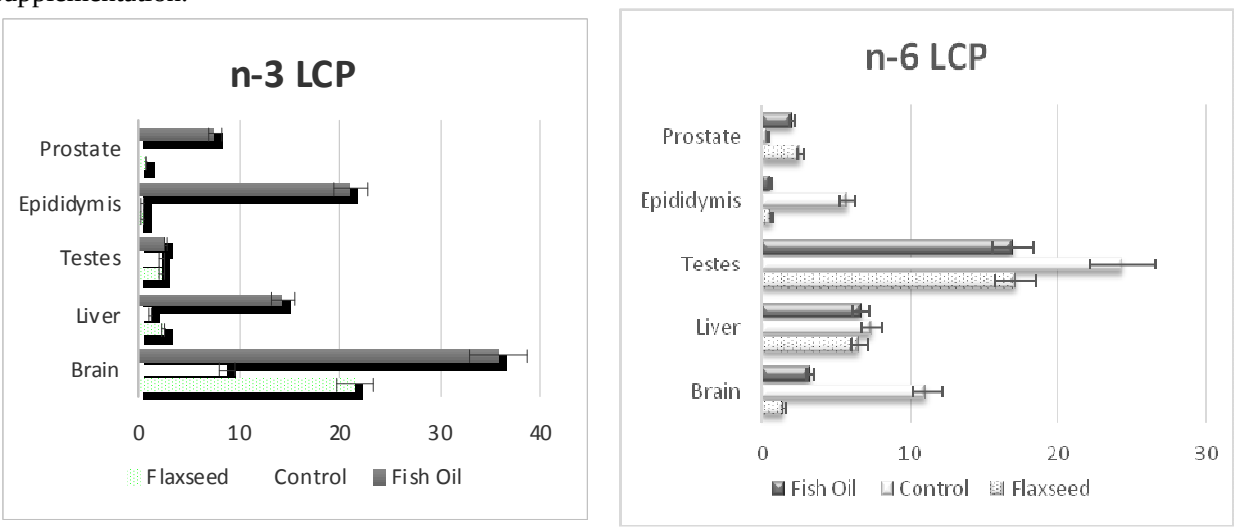
Statistical Analysis

Fatty acids profile was analyzed with a linear model evaluating the fixed effect of diet (control, flaxseed and fish oil) (STATA, 2015). Bonferroni correction was applied for multiple comparisons. The significance was set at $P \leq 0.05$.

RESULTS AND DISCUSSION

Apart testes, all the other tissues of rabbits fed fish oil showed significantly higher concentration of n-3 LCP, especially the brain (**Figure 1**). Respect to control, the dietary supplementation with flaxseed increased n-3 LCP in all the tissues. The present findings confirmed that rabbit is able to elongate and desaturase ALA even if the amount reached in the tissues remained lower than what obtained with the fish oil. On the other side, n-6 LCP were more aboundant in control group; in particular in testes, epidydimis and brain, confirming a metabolic antagonism between PUFA belonging to n-3 or n-6 series.
In fact, control diet, having a higher content of n-6 PUFA, with respect to the other diets (fish oil and flaxseed) determined a lower percentage of n-3 LCP in almost all the analyzed tissues and a higher amount of n-6 LCP.

Figure 1: n-3 and n-6 LCP in different tissues in rabbits fed standard diet (Control), fish oil and flaxseed supplementation.



It is widely reported that the distribution of n-3 and n-6 PUFA in mammalian tissues is closely linked to the diet. In mammalian brains 22:6n-3 is the major n-3LCP and generally exceeds 20:5n-3, ALA and 22:5n-3 in most lipid classes by approximately 20-fold (Sinclair, 1975). Diets rich in n-3, especially in unesterified 22:6n-3, contribute to assure proper neurological and reproductive functions, besides having positive effect on several metabolic diseases (Innis, 2007).
The phospholipids of most body tissues, including liver and testes, also accumulate n-6 PUFA mainly AA and LA. In this study, the n-3 LCP concentration in several rabbit tissues appeared modulated by the diet, according to literature. Christiansen et al. (1991) found a higher content of n-3 fatty acids in liver of rats fed fish oil compared to linseed or sunflower oil. Liver plays a central role in lipid metabolism and rapidly adapting to changes in dietary fat composition; this adaption involves changes in the expression of genes involved in glycolysis, lipogenesis, fatty acid elongation, desaturation and oxidation (Jump, 2008).
The present results corroborate the findings of the high concentration/requirement of LCP in reproductive tissues. These tissues (e.g. ovary, testis, accessory glands) are rich in PUFA and their concentration is related to essential fatty acids taken from diet; a deficiency of these PUFA can result in hypofertility (Rodríguez et al., 2019) in both male and female (Lloyd et al., 1999).

CONCLUSIONS

The present results confirm that n-3 fatty acids metabolism is affected by the n-3 dietary sources and by the antagonism exerted by n-6 ones. For this reason, when an increase in n-3 LCP is requested (Brenna et al., 2009), it is necessary to integrate diets with n-3 (precursor or derivatives). The main outcomes also

confirmed that ALA is only partly metabolised to LCP and that the n-3/n-6 ratio of feed is crucial in regulating the relative presence of n-3 or n-6 LCP in tissues. Further studies are necessary to understand the distribution of n-3 and n-6 PUFA and how to integrate these in diets to achieve a physiologically optimal level in the tissues.

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