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In Vivo Evaluation of Omega 3 Fatty Acids Fortified Infant Flours in Relation with the Growth and the Lipid Profile of Rats

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Abstract Formulated Infant flours and fortified with omega-3 fatty acids were evaluated in vivo. Three groups of five growing Wistar rats aged from 21 ± 3 days with an average weight of 43.41± 0,32 grams were fed with Omega 3 fatty acids fortified Infant flours (FMMS: Infant flour fortified with a whole soy flour and FMMHHP: infant flour fortified with a mackerel oil). Nutritional parameters (weight gain, total dry matter intake and feed efficiency ratio), the lipid profile of their serum and weight of organs (liver, spleen, brain, adipose tissue) were performed at the end of experiment. Weight gain (WG: 2.36 g / day), total dry matter intake (DMI: 10.75 g / day) and feed efficiency ratio (FE: 0.22) for FMMS and (WG: 1.63 g / day; DMI: 9. 22 g / day, FE: 0.18) for FMMHHP were higher than those of rats fed to non-fortified flour FMMHA (WG: 0.72 g / day, DMI: 7.17 g / day, FE: 0.10). The values of total cholesterol (0.64 and 0.66 mg / dl), triglycerides (0.88 and 0.98 mg / dl) and LDL cholesterol (0.05 and 0.11 mg / dl) of rats fed with fortified flours FMMS and FMMHHP were lower than those of rats fed with non-fortified infant flours (FMMHA) (total cholesterol 0.85 mg / dl, triglycerides 1.09 mg / dl, LDL cholesterol 0.24 mg / dl). The HDL cholesterol values (0.40 to 0.45 mg / dl) obtained in the rats that consumed the fortified flours was higher than value obtained in the rats that had consumed the non-fortified flour (0.32 mg / dl). The consumption of omega 3 fortified flours also resulted in a significant decrease in serum levels of total and LDL cholesterol and an increase in serum HDL cholesterol levels in the rat. The consumption of these fortified infant flours does not lead to hyperlipidemia in growing rats.

Keywords: *infant flours, fortification, omega 3, lipidemia, mackerel oil*

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1. Introduction

Malnutrition is the underlying cause of more than half of the deaths of children under five worldwide [1]. Today, more than 40 per cent of children in developing countries suffer from chronic malnutrition. This malnutrition is linked to a global deficit in the energy intake and micronutrient deficiencies. In Côte d'Ivoire, malnutrition contributes directly or indirectly to 33% of high infants and child mortality [2]. From birth to 6 months, the breast milk is the food that covers all nutritional needs of the child [3]. However, from 6 months, the breast milk becomes insufficient to cover all the energy and protein needs of the child [4]. This period is the weaning period that ranges from 6 months to 1 year or 2 years during which it is necessary to bring new foods to supplement the consumption of breast milk. By the age of 6 months, the coverage of essential fatty acid needs, which is important for the child's cognitive development, is often problematic. According to Michaelsen *et al.* [5], the breast milk is the main source of polyunsaturated fatty acids in the first two years of the life, but the content of these fatty acids in breast milk is too dependent on the mother's diet. The polyunsaturated fatty acid content of the breast milk can also be reduced in the case of inadequate maternal intakes [6]. The implementation of a strategy to fight malnutrition and infant mortality requires the enrichment of complementary foods in omega-3 polyunsaturated fatty acids. The dietary deficiency of essential fatty acids could cause kwashiorkor, exacerbating the effects of the inadequate protein intake in children suffering from malnutrition and causing skin lesions [7].

Many studies have shown that long-chain polyunsaturated fatty acids have positive effects on children's health. These molecules play a role in the development of vision [8,9]. Lau *et al.* [10], showed that the incorporation of omega 3 polyunsaturated fatty acids into the diet promotes the development of a healthy skeleton and thus later reduces the risk of developing osteoporosis. Omega-3 fatty acids also have the ability to affect bone marrow cells early in development [11]. In addition, these omega 3 fatty acids play a role in neurodevelopment and brain function [12,13]. The results of Widenhorn-Müller *et al.* [14] and Agostoni *et al.* [15] research works showed that supplementation with omega 3 fatty acids could reduce the attention deficit hyperactivity disorder in children. Increasing the consumption of omega 3 polyunsaturated fatty acids through fortified complementary foods may therefore contribute to the improved nutrition and the infant and child health. The aim of this study was to evaluate the impact of formulated infant flours fortified with omega 3 fatty acids consumption on the growth and the lipid profile of rats.

2. Materials and Methods

2.1. Materials

The animal material was composed of male Wistar albinos (*Rattus norvegicus*) rats, 21 ± 3 days old, with an average body weight of 43.41 ± 0.32 grams. These rats come from the pet shop of the nutrition and pharmacology laboratory of the Felix Houphouet Boigny University in Abidjan (Côte d'Ivoire).

The plant material was composed of three infant flours made with the millet (*Pennisetum glaucum)*, the yellow corn beans (*Zea maize*), yellow soybeans (*Glycine max),* white beans (*Phaseolus vulgaris*) and the mackerel *(Scomber scrombus)* fish oil. Two of infant flours were fortified with omega 3 fatty acids sources and one was non fortified.

2.2. Flours Production

Five (5) kg of millet, 5 kg of yellow corn, 5 kg of white beans and 5 kg of yellow soybeans were used to produce flours. The millet and corn beans were sorted, washed, soaked for 48 hours and then spread on a damp cloth for 3 days. Sprouted beans were dried, degermed and milled using a hammer mill (FORPLEX, France) to obtain sprouted corn and millet flours. White beans and soy beans were sorted, washed, soaked for 3 hours, drained and then dried. The seeds were then roasted, manually degermed and milled in flour using a hammer mill (FORPLEX, France).

2.3. Extraction of Fish Oil

The enzymatic hydrolysis was performed according to Gbogouri *et al.* [16] adapted method. 500g of fresh mackerel fish crushed (*Scomber scrombus*) was suspended in distilled water and put in a stirred thermostated reactor (2 L). The adjustment of pH with NaOH 4 N was done for 15 min under mixing. The enzyme solution was then added and the reaction allowed for 2h under nitrogen and constant agitation at 600 rpm. The pH was kept constant by automatically adding NaOH 4 N during hydrolysis according to the pH-stat method. The volume of NaOH was recorded to allow calculation of the degree of hydrolysis (DH). Enzymes were inactivated in the hydrolysates by the heat treatment with microwave at 95℃ /5 min. The medium was coarsely filtered to retain bones, whereas the liquid phase was subjected to subsequent centrifugation to separate the oil, the emulsion fraction and the sludge from the underlying aqueous phase.

The supernatant oil fraction after centrifugation was removed with a pipette and stored at -20 ° C after addition of 0.02% vitamin E (antioxidant).

2.4. Formulation of Flours

Flours were prepared by blending the individual ingredients using the matrix method of formulation according to Olusayo *et al.* [17] adapted method. This lead to generate proportions of ingredients to be mixed to obtain required nutrient content (Table 1).

Table 1. Composition of Formulated Flours

Ingredients	Proportions (g/100g)		
	FMMHA	FMMS	FMMHHP
Millet flour	68	68	54
Corn flour	10	10	8
White bean flour	16	00	30
Soybean flour	00	16	00
Mackerel oil	00	00	3
Sugar	6	6	5

FMMHA: Millet-corn-white beans Flour
FMMS: Millet-Corn-Soybean flour
FMMHHP: Millet-Corn-white bean-Fish Oil flour

2.5. Biochemical Analysis of Formulated Flours

2.5.1. Proximate Composition

Nutrient components of flours (dry matter, fibbers, proteins, fatty matters) were determined according to AOAC method [18].

The carbohydrate content was determined by the difference in nutrients using the following equation:

$$\%\ carbohydrates = \left[100 - \begin{pmatrix} \%\ Moisture + \%\ Proteins \\ +\ \%\ Lipids + \%\ Ash + \%\ Fibres \end{pmatrix}\right]. \quad (1)$$

The energy value was calculated according to Livesey method [19] and expressed on the dry matter basis using the following equation:

$$Energy\ value = (\%\ Proteins \times 4) + (\%\ Carbohydrates \times 4) + (\%\ Lipids \times 9). \quad (2)$$

2.5.2. Fatty Acid Composition

The fatty acid composition of the flours was determined by gas chromatography. The fatty acid methyl esters of the

lipid fractions of the fish were prepared according to the AOAC method [18]. The transmethylation was carried out using 1 ml of BF3 in methanol (14%, w / v) and 1 mL of toluene at 100°C. After extraction of the methyl esters of fatty acids with cyclohexane, they were washed with Distilled water and analyzed by gas chromatography (CG-2010 plus, Shimadzu) with a flame ionization detector and a 30 m capillary column long with a film thickness of 0.25 µm, an internal diameter of 0.32 mm. The oven temperature was set at 200°C, the sensor and injector temperatures were 250°C. The nitrogen pressure used as the inlet carrier gas ranged from 6.90 to 47.6 KPa. The flow rate was maintained at 1 cm / min and the dead time was 1 min 15 s (hydrogen 40 cm / s). Fatty acid methyl esters (PUFAs 1 and PUFAs 2 from marine sources, Supelco, Sigma-Aldrich, and Bellefonte, PA, USA) have been used as standards for the identification of fatty acids. The percentage of methyl esters of fatty acids was calculated from the total area of all peaks. The results were presented as a percentage of the total fatty acids identified.

2.6. Animal Experimentation

The rats were distributed on the basis of their more or less homogeneous weight in three lots of five rats in individual metabolic cages. The rats were fed according to the method of Adrian *et al.* [20] modified and taken up by Gernah *et al.* [21]. The experiment lasted 31 days including three days of adaptation during which the rats were fed only with pellets manufactured by the company "IVOGRAIN" (Abidjan). Three (3) flours were tested including one non-fortified flour (FMMHA) and two fortified flours (FMMS and FMMHHP) respectively with soybean flour and mackerel fish oil. A group of 5 rats were fed with non-fortified flour (FMMHA), a lot of 5 rats were fed with the flour fortified with the whole soybean flour (FMMS) and another lot of 5 rats were fed with the mackerel fish oil fortified flour (FMMHHP). Flours were distributed *ad libitum* once a day (in the mornings at 8 am). Before their distribution, the various flours were reconstituted into paste with water in order to reduce the waste. The water was served at will and renewed every three days. The dry matter of the pasta was measured daily on samples taken for this purpose. The next day, the remains were collected and their dry matter was determined. The animals were weighed at the beginning of the experiment and then at two-day intervals with a Sartorius scale (precision: 0.001 g). The last weighing took place at the end of the experimental period. The growth was determined by the difference between the initial weight and the final weight. The difference between quantities of food served and the amount of food left referred to the dry matter made it possible to determine the consumed quantity.

2.7. Collection of Blood Samples and Organs

Rats were fasted for at least 12 hours at the end of the feeding period. They were anesthetized with ethyl urethane (20 %) and then sacrificed by decapitation. The blood was collected in dry tubes. These tubes were centrifuged in a refrigerated centrifuge (4°C) (EPPENDORPH, Germany), and the collected serum was used for the determination of blood lipid parameters. After the blood collection, longitudinal laparotomy was performed on rats, to remove the liver, spleen, brain and adipose tissue in the abdomen. These organs were rinsed with physiological solution (NaCl 9‰), dehumidified on clean paper towels, and weighed on a Sartorius scale (precision: 0.001 g). The weight of the organs was expressed as a percentage of the live weight of the animal, obtained during the last weighing. The relative organs body weights were calculated using the formula below.

$$\text{Relative organ body weight } \% = \frac{\text{Weight of the organ } (g)}{\left[\begin{array}{l}\text{Weight of the animalat} \\ \text{the end of the experiment}(g)\end{array}\right]} \times 100 \quad (3)$$

The effects of consumption of omega 3 fortified flours on growth parameters were measured. The weight gain (WG) and the total dry matter intake (DMI) were estimated per day. The Table 2 shows mathematical expressions of nutritional parameters.

Table 2. Expression of nutritional parameters

Nutritional parameters	Mathematical expressions
Weight gain (W.G) (g)	Final weight - initial weight
Weight gain (W.G) (g/j)	Final weight - initial weight/number of days
Total dry matter intake (DMI) (g)	Total dry matter (of the food) intake during the experimentation period
Total dry matter intake (DMI) (g/j)	DMI (g) / number of days
Coefficient of food efficiency (FE)	W.G (g) / DMI (g)

2.8. Determination of Blood Lipid Parameters

The total cholesterol, triglycerides and the HDL cholesterol were measured using enzymatic methods on the biochemistry auto-analyzer (Mindray BA-88 A, China). Values obtained after the determination of the total cholesterol, triglycerides and the HDL cholesterol, made it possible to calculate the value of the LDL cholesterol in each serum by the formula of Friedewald *et al.* [22].

$$C - LDL\ (mg/dl) = total\ Cholesterol\ (mg/dl) - \left[\begin{array}{l}C - HDL\ (mg/dl) \\ +TG/5\ (mg/dl)\end{array}\right] \quad (4)$$

Where:
TG: triglycerides
C-LDL: LDL cholesterol
C-HDL: HDL cholesterol.

2.9. Statistical Analysis

All data obtained from the experiments were statistically analyzed using one-way Analysis of Variance (ANOVA) and means separated by Duncan's Multiple Test. The significant differences between means were determined by Least Significant Difference (LSD) test. Significant difference was accepted at 5 % level of probability.

3. Results

3.1. Proximate Composition

Table 3 showed proximate composition of fortified flours. The moisture content of the flours was 3.12 and 2.12% for FMMS and FMMHHP, respectively. Ashes, lipids, proteins, fibers and carbohydrates levels were 2.76 and 2.45%; 11.75 and 12.29%; 13.50 and 14.41%; 3.02 and 4.61%; 65.85 and 64.13% for FMMS and FMMHHP, respectively. The energy supplied by FMMS and FMMHHP was 423.19 and 425.75 kcal / 100g, respectively.

Table 3. Proximate composition of formulated flours

Component	FMMS	FMMHHP
Moisture (%)	3,12 ± 0,04^{a}	2,12 ± 0,01^{b}
Ash (%)	2,76 ± 0,04^{a}	2,45 ± 0,01^{b}
Lipids (%)	11,75 ± 0,33^{b}	12,29 ± 0,22^{a}
Proteins (%)	13,50 ± 0,07^{b}	14,41 ± 0,03^{a}
Fibres (%)	3,02 ± 0,02^{b}	4,61 ± 0,12^{a}
Carbohydrates (%)	65,85 ± 0,50^{a}	64,13 ± 0,01^{b}
Energy value (Kcal/100 g)	423,19 ± 1,46^{b}	425,75 ± 0,99^{a}

FMMS: Millet-Corn-Soybean flour
FMMHHP: Millet-Corn-white bean -Fish Oil flour
In line, mean values with different superscript differed significantly (Duncan test, $p \leq 0.05$).

3.2. Fatty Acid Composition of Flours

The fatty acid composition of the flours is shown in Table 4. The FMMS flour contained 8 total fatty acids compared to the FMMHHP flour which contained 13 total fatty acids. FMMHA, FMMS and FMMHHP flours contained the three major groups of fatty acids. These were saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The saturated fatty acid contents of non-fortified (FMMHA) and fortified (FMMS and FMMHHP) flours were about 1630 mg, 1030 mg and 1370 mg per 100 g of flour, respectively. The monounsaturated fatty acid content of non-fortified flour (FMMHA) and fortified flours (FMMS and FMMHHP) were 1310 mg, 1450 mg and 2710 mg per 100 g of flour, respectively. The polyunsaturated fatty acid content of non-fortified flour (FMMHA) and fortified flours (FMMS and FMMHHP) were 2830 mg, 4360 mg and 3660 mg per 100 g of flour, respectively. Omega 3 fatty acid contents of non-fortified flour (FMMHA) and fortified flours (FMMS and FMMHHP) were 180 mg, 490 mg and 2720 mg, respectively. For omega 6 fatty acid, contents were 2650 mg, 3870 mg and 940 mg per 100g of Non-fortified flour (FMMHA) and fortified flours (FMMS and FMMHHP), respectively. The n-6 PUFA / n-3 PUFA ratio of non-fortified flour FMMHA was 14.72. The n-6 PUFA / PUFA n-3 ratio of fortified flours was 7.90 and 0.35 for FMMS and FMMHHP, Respectively.

3.3. Effects of Omega-3 Fortified Flours on the Growth Characteristics of Rats

As shown in Table 5, the weight gain (WG), the total dry matter intake (DMI) and the feed efficiency ratio (FE) indicated the effect of fortified flour consumption in the rat. For these 3 parameters (WG, DMI, FE), there was a significant difference between fortified flours and non-fortified flours ($p< 0.05$). The weight gain was about 2.36 g / day for FMMS, 1.63 g / day for FMMHHP and 0.72 g / day for FMMHA. The total ingested dry matter was 10.75 g / day for FMMS, 9.22 g / day for FMMHHP and 7.17 g / day for FMMHA. The feed efficiency coefficient was 0.22 for the FMMS, 0.18 for the FMMHHP and 0.10 for the FMMHA.

Table 4. Fatty acids content of flours

Fatty acids content (mg/100g of flour)			
Fatty acids	**FMMHA**	**FMMS**	**FMMHHP**
C14 :0	trace	10^{b}	370^{a}
C16 :0	1240^{a}	750^{b}	830^{c}
C16 :1	trace	trace	690^{a}
C18 :0	330^{a}	250^{b}	170^{c}
C18 :1n9	1310^{b}	1370^{b}	1500^{a}
C18 :1n7	trace	80^{b}	320^{a}
C18 :2n6	2650^{b}	3870^{a}	150^{c}
C18 :4n3	180^{c}	490^{b}	1090^{a}
C20 :0	60^{a}	20^{b}	trace
C20 :1n9	trace	trace	200^{a}
C20 :4n6	trace	trace	790^{a}
C20 :5n3 (EPA)	00	00	750^{a}
C22 :0	trace	00	trace
C22 : 5n3	trace	trace	90^{a}
C22 :6n3 (DHA)	00	00	790^{a}
Total SFA	1630^{a}	1030^{c}	1370^{b}
Total MUFA	1310^{c}	1450^{b}	2710^{a}
Total PUFA	2830^{c}	4360^{a}	3660^{b}
Total PUFA n-3	180^{c}	490^{b}	2720^{a}
Total PUFA n-6	2650^{b}	3870^{a}	940^{c}
Total PUFA n-6/total PUFA n-3	14,72^{a}	7,90^{b}	0,35

FMMHA: Millet-Corn-white bean flour; FMMS: Millet-Corn-Soybean flour; FMMHHP: Millet-Corn-white bean-Fish Oil flour.
SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid.
In line, means with different superscript differed significantly (Duncan test, $p \leq 0.05$).

Table 5. Effects of omega-3 fortified flours on the growth characteristics of rats

Parameters	Flours		
	FMMHA (n=5)	FMMS (n=5)	FMMHHP (n=5)
Initial weight (g)	46.33± 5.20^{a}	40.05± 4,79^{a}	43.84 ± 5,94^{a}
Final weight (g)	66.49 ± 4.27^{c}	106.13±13.81^{a}	89.48 ± 7.73^{b}
WG (g/j)	0.72± 0.12^{b}	2.36± 0.55^{a}	1.63± 0.40^{b}
DMI (g/j)	7.17± 0.60^{b}	10.75± 1.20^{a}	9.22± 0.45^{ab}
FE	0.10± 0.01^{b}	0.22± 0.02^{a}	0.18± 0.01^{a}

FMMHA: Millet- Corn-white beans flour; FMMS: Millet-Corn-Soybeans flour; FMMHHP: Millet-Corn-white bean-Fish Oil flour; WG: weight gain; DMI: Dry Matter Intake; FE: feed efficiency
Values are the mean ± standard deviation of five rats. In line, mean values with different superscript differed significantly (Duncan test, $p \leq 0.05$).

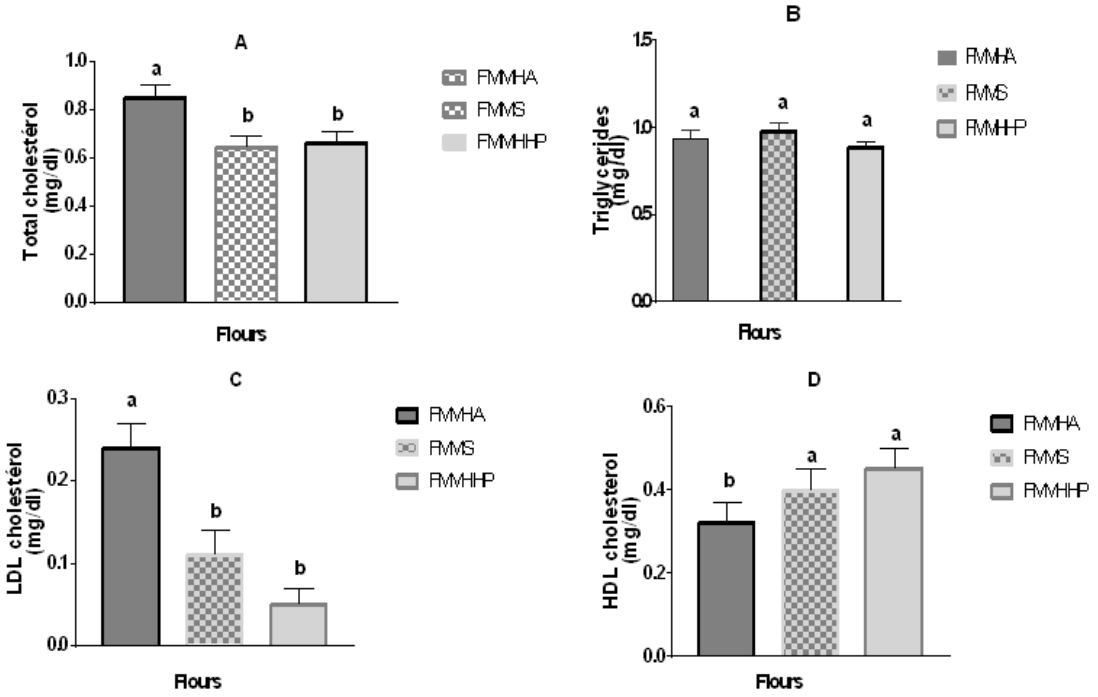

Figure 1. Composition of rats' plasma in Total cholesterol (A), Triglycerides (B), LDL-cholesterol (C) and HDL-cholesterol (D)

3.4. Effects of Omega-3 Fatty Acids Fortified Flours on Blood Lipid Parameters

Figure 1 shows that the level of the serum total cholesterol levels were 0.85, 0.64 and 0.66 mg/dL in FMMHA fed rats and in fortified flours (FMMS and FMMHHP)-fed rats, respectively. Serum triglyceride levels were 0.93, 0.98, and 0.88 mg / dL in FMMHA-fed rats and in fortified flours (FMMS and FMMHHP)-fed rats, respectively. Serum HDL cholesterol levels were 0.32, 0.40 and 0.45 mg / dL in FMMHA-fed rats) and in FMMS and FMMHHP-fed rats, respectively. Serum LDL cholesterol levels were 0.24, 0.11, and 0.05 mg / dL in FMMHA -fed rats and in fortified flours (FMMS and FMMHHP) fed rats, respectively. Flours fortified in omega 3 fatty acids (FMMS and FMMHHP) lead to a significant decrease in serum total cholesterol and LDL cholesterol levels. No significant variation in serum triglyceride levels was observed in rats irrespective of the flour. The consumption of flours fortified with omega 3 fatty acids leads to a significant increase in serum HDL cholesterol levels in rats. There is a significant difference between serum total cholesterol, LDL cholesterol, and HDL cholesterol levels in fortified flours-fed rats (FMMS and FMMHHP) and in non-fortified flour-fed rats (FMMHA) at the 5% threshold.

3.5. Effects of Omega-3 Fortified Flours on Organs Weight

The mean relative weights of liver, spleen, brain, and abdominal adipose tissue in rats are shown in Figure 2. The mean relative weights liver of rats fed with fortified flours (FMMS and FMMHHP) and non-fortified flour (FMMHA) were 3.20, 3.46 and 3.19% respectively. The mean spleen weights of fortified flours (FMMS and FMMHHP) fed rats and non-fortified flour (FMMHA) -fed rats were 0.31, 0.30 and 0.27%, respectively. The mean brain weights of rats fed with fortified (FMMS and FMMHHP) and non-fortified (FMMHA) flours were 1.57, 2.15 and 1.36%, respectively. There is no significant difference between the relative brain weight of rats fed with fortified flour FMMS and the relative brain weight of rats fed with non-fortified flour FMMHA. However, significant brain weight variation was observed in rats fed with flour fortified with fish oil FMMHHP. The relative weights of adipose tissue in rats fed with fortified (FMMS and FMMHHP) and non-fortified (FMMHA) flours were 1.19, 0.92 and 0.91%, respectively. The relative weight of adipose tissue in rats fed with flour FMMS fortified with soybeans flour (1.19%) was greater than the relative weight of adipose tissue in rats fed with the non-fortified flour FMMHA (0.91%) and the flour FMMHHP fortified with fish oil (0.92%). Statistical analysis shows that there is a significant difference at the 5% level between the relative weight of adipose tissue in rats fed with FMMS flour and the relative weight of adipose tissue in rats fed with FMMHA and FMMHHP flours.

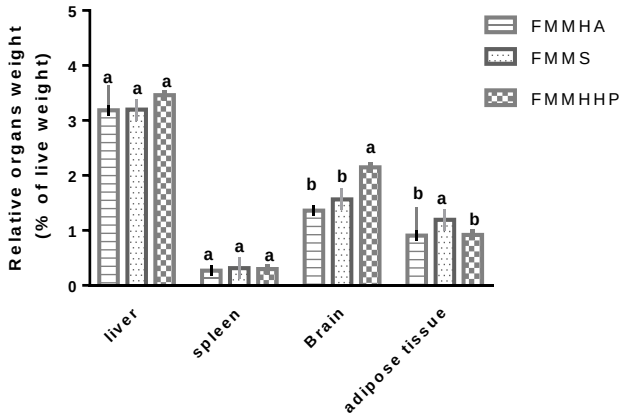

Figure 2. Relative organs weight of rats fed non enriched and enriched flour with omega 3 fatty acids

4. Discussion

Carbohydrate, lipid and protein contents of flours were in accordance with the nutritional recommendations [23]. The use of cereal and legume (such us whole soybean) flours and fish oil in the formulations could explain the macronutrient levels. According to Singh *et al.* [24] and Temesgen *et al.* [25], the mix of roots, tubers and/or cereals and legumes are the main sources of carbohydrates and proteins in the diet. The studied flours were designed to provide 400 kcal / 100 g of flour as recommended by the World Health Organization. The energy intake of a food is the result of energy related to macronutrients (carbohydrates, proteins and lipids). The consumption of flours could prevent protein-energy malnutrition in children.

Fortified flours FMMS and FMMHHP have high levels of polyunsaturated fatty acids and omega 3 fatty acids compared to non-fortified flour FMMHA. The high levels of polyunsaturated fatty acids and omega 3 fatty acids could be explained by the addition of the whole soy flour and the mackerel fish oil, both were sources of polyunsaturated fatty acids and omega 3 fatty acids. FMMHHP flour has an omega 3 fatty acid content higher than the omega 3 fatty acid content of non-fortified flour FMMHA and fortified by the addition of complete soy flour FMMS. This higher content of omega 3 fatty acids in fish oil enriched flour may be attributed to the presence of more omega 3 fatty acids in fish oils [26]. FMMHA and FMMS flours have high omega 6 fatty acid content compared to the omega 6 fatty acid content of FMMHHP flour. According to Combe and Boué-Vaysse [27], soybean oil contains low levels of omega-3 fatty acids (4 to 10%) and is an important source of omega-6 fatty acids (50 to 62%). The presence of long chain omega 3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the FMMHHP flour was due to the presence of these fatty acids in mackerel fish oil. According to Kris-Etherton *et al.* [28], fish oils are the only major dietary source of EPA and DHA.

Concerning the impact of the consumption of omega 3 fatty, the weight gain in rats fed mackerel-enriched flour was lower than in rats fed with the soy enriched and unenriched flour. This result would be due to the high level of omega 3 fatty acids in fish oil enriched flour. Indeed, according to Simopoulos [29], a high intake of omega-3 fatty acids in the diet would reduce the risk of weight gain. This result is in agreement with other works who found that diets high in omega 3 fatty acids content resulted in a reduction in visceral fat in rats and a decrease in size and number of adipocytes [30,31]. In addition, this result could be attributed to the presence of long-chain polyunsaturated fatty acids (eicosapentaenoic acid and docosahexaenoic acid) in fish oil. According to Hill *et al.* [32] and Hun *et al.* [33], diets high in n-3 polyunsaturated fatty acids content, such as those found in fish oils, tend to reduce weight gain compared to other dietary oils.

Concerning the consumption, the differences between the quantities of food consumed in the form of dry matter (DMI) by the rats fed with the flours are significant. The ingredients used for the formulation may partly explain these small differences in the amount of food consumed. According to Borys [34], food consumption depends on several factors including the physiological state of the body as well as factors related to food characteristics such as aroma, flavour and chemical composition.

The significant decrease in serum levels of total cholesterol and LDL cholesterol in omega-3 fortified flours fed rats could be attributed to the cholesterol-lowering effect of soy and fish oil. Omega-3 fatty acids which are present in soybeans are thought to be responsible for lowering serum levels of cholesterol and LDL in rats [35]. Furthermore, eicosapentaenoic acid (EPA) is the hypotriglyceridemic fatty acid of fish oil. EPA inhibits synthesis and secretion of triacylglycerol by the liver in contrast to DHA [36].

The results showed an increase in HDL cholesterol levels in the blood of rats fed with fortified flours compared to rats fed with non-fortified flour. The increase in HDL cholesterol could be explained by the effect of omega 3 fatty acids brought by the fish oil and the soybean oil. The enrichment of omega 3 fatty acids leads to an increase in HDL cholesterol. According to François *et al.* [37], the increase of HDL cholesterol is due to a significant reduction of total cholesterol by omega 3 fatty acids.

The increased feed efficiency ratio (FE) for the flour fortified with whole soybeans flour (FMMS) could be explained by a better assimilation of this flour by rats. The consumption of infant flours fortified in omega 3 fatty acids by the addition of mackerel fish oil and soy flour does not influence the weight of the liver and spleen of rats. However, the consumption of flour fortified by the addition of whole soy flour resulted in a significant increase in adipose tissue weight in rats. The omega 3 fatty acid content of soy flour may justify the increase in adipose tissue weight in rats. Soy flour is a source of omega 3 fatty acids. However, like most oilseeds, its omega 3 fatty acid content is low compared to that of fish oils. This low content would have caused an increase in adiposity in rats compared to that of fish oil. According to Ukropec *et al.* [38], a low omega 3 fatty acid content in the diet increases fat deposits in adipose tissue by increasing the activity of lipogenic enzymes and decreasing beta-oxidation. The consumption of flour fortified with fish oil caused an increase in the brain size of the rats. This increase in brain size could be explained by the presence of long-chain n-3 PUFAs (EPA and DHA) in the mackerel oil. Several studies have shown that long-chain omega-3 polyunsaturated fatty acids stimulate brain cell proliferation, neurite outgrowth, and synaptogenesis in rats [39,40,41,42].

5. Conclusion

The results of this study showed that the consumption of infant flours fortified by the addition of mackerel oil (source of omega 3 fatty acids) did not cause significant weight variations in growing rats. Fortification by adding mackerel oil reduces the risk of obesity in young rats, as opposed to the consumption of flour fortified by the addition of whole soy flour. The consumption of omega 3 fortified flours also resulted in a significant decrease in serum levels of total and LDL cholesterol and an increase in serum HDL cholesterol levels in the rat. The consumption

of these fortified infant flours does not lead to hyperlipidemia in growing rats. With respect to organ biometrics, consumption of these fortified flours does not lead to enlarged liver and spleen in the rat. However, it leads to good brain development in growing rats. These data may suggest that the consumption of omega 3 fortified flours may have beneficial effects on the health of growing rats and, consequently in children.

Statement of Competing Interests

The authors have no competing interest in relation to their work.

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