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Original article

The possible effect of diets containing fish oil (omega-3) on hematological, biochemical and histopathogical alterations of rabbit liver and kidney

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ABSTRACT

The dietary intake of omega-3 polyunsaturated fatty acids has emerged over the past 20 years as an important way to modify cardiovascular risk. The present study aimed to evaluate the possible effects of a partial replacement of soybean meal in control economic diet by different concentrations of fish oil on the possible harmful changes in histological structure of liver and kidney and blood parameters in rabbits. A total of 36 adult New Zealand rabbits were equally divided into four groups, (control diet and control diet supplemented with different concentrations of fish oil at levels of 0.5, 1.0 and 1.5 mL fish oil per day/kg live body weight) and dissected after 6 weeks. Our results showed that, feeding diet supplemented with fish oil were significantly increased the percentages of hemoglobin, platelets, the mean corpuscular hemoglobin, WBCs count, total proteins, albumin, albumin/globulin ratio, $_S$GOT and testosterone and significantly decreased the total lipids, cholesterol and triglycerides. The used of fish oil are good supplements for growing rabbits without any adverse effect on histological structure of liver and kidney in rabbits.

1. Introduction

Because of increasing the cost of animal feed ingredients as well as the high demand, especially for the protein supplements, several efforts were carried out to use untraditional feed protein ingredients to participate in facing feed shortage problem and at the same time to decrease feeding costs [1,2]. Lipids are important nutrients, providing 25–45% of dietary energy in most affluent societies, storage and transport of energy, for insulation and for mechanical protection [3]. In addition, lipids provide polyunsaturated fatty acids that are essential nutrients of importance for several cellular functions in the body including ligands for transcription factors, precursors of signal molecules and building blocks in all cells of the body [4,5].

Fat is a macronutrient that is considered to be a major source of calories or energy [6,7]. Although some fat in the diet is necessary, too much of fat can lead to heart diseases, cancers, obesity and other health problems [8]. They can be saturated, monounsaturated or polyunsaturated. Saturated fatty acids contain no double bond, monounsaturated one double bond and polyunsaturated contains two to six double bonds. The polyunsaturated fatty acids of four to six double bonds are a characteristic for fish oil, resulting in the unique health property often referred as the omega-3 fatty acids [5]. Long chain and intermediate chain fatty acids must be consumed as part of the diet because they cannot be synthesised by humans [9]. Investigations of fish oils have not only shown their importance as a dietary source of vitamins A and D, but also that they are very rich in fatty acids of long chained omega-3 [10]. It is not known which biological effects of omega-3 fatty acids are essential, but it is possible that the omega-3 fatty acid-derived eicosanoids are crucial, as well as the unique structural properties of omega-3 fatty acids for cell membranes [11].

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Recently, several efforts were carried out to use natural materials as fish oil by-products in animal feeding due to their participation as part in the solution of feed shortage problems and dramatic increases in prices of animal feed ingredients [6,12,13]. Based on these facts, it is no surprise that omega-3 fatty acids also have beneficial effects on many biological systems, including immune reactions, blood platelets, smooth muscle cells, endothelial cells, liver cells, heart cells, adipocytes, osteoblasts and neurons. They may also promote regulation of growth and apoptosis in several different cell types [4]. Fish and other marine animals, and oils from these sources are rich in omega-3 fatty acids, and they have been important ingredients of the human diet for many populations during thousands of years [5,14]. Numerous investigations have shown that consumption of fish or dietary supplementation of fish oils rich in long chain omega-3 polyunsaturated fatty acids, not only lowers the risk of cardiovascular and coronary heart disease [5,7], but can also inhibit the development of cancers, stimulates immune functions [15] and helps the development of the brain [16]. Rabbits rank fifth in the world production of meat, but the importance of rabbit meat is increasing because of its high dietetic, taste and prohealth value. Compared to meat of other species it is characterized by low cholesterol level and high level of linolenic acid, and should therefore be included in the human diet [2,6,13]. Many researchers shown the effectiveness of fish oil in growing rabbit diets on nutrients digestibility, feeding values, nitrogen balance and productive performance without had shown the blood plasma analysis and histopathological effects of diets on rabbit organs [2]. Therefore, the present study was designed to investigate the possible physiological and histopathological effectiveness of diet supplemented with different concentrations of fish oil in growing rabbit diets in addition to the changes on some blood parameters.

2. Material and methods

The present study was conducted at a rabbit farm in Agriculture Experiments and Researches Station, Poultry Production Farm, Faculty of Agriculture, Mansoura University, Egypt, during March and May 2012. A total number of 36 growing New Zealand white (NZW) male rabbits at 7 weeks of age (with an average weight about 960 ± 30 g) were kept under the same managerial and hygienic conditions. The rabbits were housed in galvanized wire cages (50 × 60 cm) provided with feeds and automatic nipple drinkers. Feed and water were offered *ad libitum*. Rabbits were individually weighed and randomly distributed into four groups of nine rabbits:

- G_1: control group were rabbits fed on economic diet without any supplementation (Table 1);
- G_2: rabbits fed on economic control diet supplemented with 0.5 mL fish oil per day/kg live body weight;
- G_3: rabbits fed on economic control diet supplemented with 1.0 mL fish oil per day/kg live body weight;
- G_4: rabbits fed on economic control diet supplemented with 1.5 mL fish oil per day/kg live body weight.

Each 10 mL fish oil contained 1000 mg cod liver oil, flavor rich in omega-3 fatty acids, vitamin A and vitamin D. After 6 weeks of experiment, the animals were fasted for 10 h and dissected after euthanized with intraperitoneal injection with sodium pentobarbital and subjected to a complete necropsy.

Blood samples were individually collected from the inferior vena cava of each rabbit in heparinized and non-heparinized glass tubes. Complete blood picture (CBC) were shown from the collected blood samples by automatic methods (Sysmex kx-21n automated hematology analyzer; JAPAN CARE CO., LTD). Blood serum was separated by centrifugation at 3000 rpm for 15 minutes. The collected serum was stored at −18 °C. Total protein was measured using diagnostic kit according to Henery [17]; albumin was measured using diagnostic kit according to Drupt [18]. The serum globulin was calculated by subtracting the value of albumin from the value of total protein according to Doumas and Biggs [19]. Total lipids according to Esher et al. [20]; serum total cholesterol was determined according to the method of Allain et al. [21] and serum triglycerides were determined according to the method of Fossati and Prenciple [22] using kits supplied by Human. Liver functions as AST (sGOT) and ALT (sGPT) were determined according to Rietman and Frankle [23]; urea was measured using diagnostic kit according to Fawcett [24] and creatinine according to Bowers and Wong [25]. Determination of T_3, T_4 and TSH was performed using Biocheck Kits Inc. (USA) catalog no BC-1005, BC-1008 and BC-1001 respectively (using monoclonal antibody). Testosterone levels were assayed using the same RIA kit according to Tietz [26].

Table 1
The composition of the dietary treatments (diets were manufactured in pellets shape 4 mm diameter at El-Safwa Factory, Daqahleia, Egypt).

Ingredients, %	Treatments			
	G_1	G_2	G_3	G_4
Yellow corn	10	10	10	10
Wheat bran	25	25	25	25
Barley grain	14.6	14.6	14.6	14.6
Soybean meal	15.5	15.0	14.5	14.0
Berseem hay	30	30	30	30
Molasses	3	3	3	3
Salt	0.4	0.4	0.4	0.4
Limestone	1	1	1	1
Premix	0.5	0.5	0.5	0.5
Fish oil	0	0.5	1.0	1.5

G: group.

Specimens of liver and kidney were then immediately removed, sliced sagitally and fixed in 10% neutral-buffered formalin for 24 hours. The specimens were then dehydrated, cleared and embedded in paraffin. Serial sections of 5 μm thick were cut by mean of rotary microtome (Litz). Sections were processed for haematoxylin and eosin staining according to Bancroft and Stevens [27].

Statistical analysis: data were expressed as mean values ± SE and statistical analysis was performed using one way ANOVA to assess significant differences among treatment groups. The criterion for statistical significance was set at $P < 0.05$ for the biochemical data. All statistical analyses were performed using SPSS statistical version 16 software package (SPSS® Inc., USA).

3. Results

Blood constituents as affected by the different experimental diets are shown in Table 2. The percentages of hemoglobin, platelets, mean corpuscular volume (MCV) and white blood cells count were significantly increased in experimental groups (G_2, G_3 and G_4) when compared with control group (G_1). Also, RBCs and hematocrate were significantly increased in G_2 and G_3 when compared with G_1 while in G_4 significantly decreased in when compared with G_2 and G_3. No changes in the mean corpuscular hemoglobin concentration (MCHC) levels with different groups when compared with G_1.

Table 3 shows that serum total proteins in rabbits blood serum and their fractions (albumin and globulin) as indicator of protein