<u>Original Article</u>

Influence of the Oral Administration of Fish Oil on Hematological and Biochemical Performance in Local Male Rabbits

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Abstract

In some developing countries in Africa, Asia, and Latin America, rapid population growth is a complicated problem, and thus, it is necessary to raise the efficiency of foodstuff production to preserve human life. Therefore, this study was designed to investigate the effect of fish oil supplementation on male rabbits' productivity, as well as their biochemical and physiological characteristics. This experiment was conducted in the Animal House of the College of Veterinary Medicine, University of Baghdad (Baghdad, Iraq) for eight weeks. Twenty-four local male rabbits were randomly divided into three groups (each containing eight rabbits) as follows: the control group that was fed with a basal diet only, the first treated group in which each animal received fish oil orally at a dose of 0.75 ml every day, and another treated group in which each animal received fish oil orally at a dose of 1.5 ml every day. Both treated groups received the treatment for 60 days. Blood samples were collected from cardiac veins by cardiac puncture at the beginning of the experiment (Zero time). On day 60 of the experiment, samples were collected again to analyze potential changes in blood characteristics, including white blood cells count, hemoglobin, and red blood cells count. These blood samples recorded a significant decrease in cholesterol in the two treatment groups, compared to the control. In conclusion, the current findings recommend daily oral administration of fish oil at a dose of 0.75 mg/ml or 1.5 mg/ml to rabbits for 60 days before conception to improve performance trials, as well as biochemical and hematological results. These alterations, however, have a small impact on these features, compared to higher doses administered to rabbits.

Keywords: Biochemical tests, Fish oil, Hematological tests, Male rabbits, Omega-3, Productive features

1. Introduction

In some developing countries in Africa, Asia, and Latin America, rapid population growth is a complicated problem, and thus, it is necessary to raise the efficiency of foodstuff production to preserve human life (1, 2). Therefore, there is a growing interest in farm animals, such as rabbits, and their production as they play an essential role in the strategy of human food security in these countries (3). Rabbits, as mono-gastric animals, have unique features, such as rapid reproduction and frequent births during a year with a large number of newborns each time (4). These characteristics are due to the speed of growth, the short period of pregnancy, their ability to inseminate and fertilize immediately after birth, as well as other reproductive characteristics, including early puberty, which occurs at the age of five months among females and males (2, 5).

Omega-3 (also called n-3), which exists mainly in fish oil, is one of the most important food additives due to its health benefits (6). It is one of the essential fatty acids for the body provided through simple and natural metabolic processes. Omega-3 is also necessary for the growth of humans and animals to have a healthy body. It belongs to the polyunsaturated fatty acid family with members, including linolenic, docosahexaenoic, and eicosapentaenoic acids (7). Furthermore, it is possible to add other feedstuff to the rabbit diet to increase digestion efficiency and the body's ability to benefit from its materials and compounds, such as raw fat (8). In general, the rabbit diet contains grains, coarse materials, and some green fodder; however, several supplements can fortify it (9).

Experiments have identified several crucial characteristics of rabbit lipid metabolism similar to those reported in humans. Compared to mice and rats, higher levels of Apolipoprotein B have been identified in rabbits. It is well-documented that rabbits are also susceptible to developing atherosclerosis. These physiological features make them a suitable experimental animal model for translational studies on obesity-related disorders, growth performance, and productivity (10). Biochemical blood tests can identify and diagnose some disorders in these animals, which can provide evidence of their health status (10). It is crucial to carefully formulate raw fat proportion in the rabbit diet to avoid problems in the digestive system, as well as vital organs, especially the kidneys and liver (11).

Therefore, this study aimed to investigate the effect of fish oil supplementation on male rabbits' productivity and their biochemical, as well as physiological, characteristics.

2. Materials and Methods

2.1. Animals and Feeding

Two weeks before the experiment, the animals were housed under controlled environmental conditions (20°C±2°C,14:10 h light-dark cycle) with ad libitum access to food and water, and they were administered prophylactic coccidiosis. In the beginning, the animals were fed mainly on the forage brought from the college farm and were provided with concentrate (grower diets) (12).

Twenty-four local male rabbits $(1.10-1.42\pm0.1 \text{ kg})$ at the age of 4-5 months were used in this study, and each animal was in a single cage. The animals were randomly divided into three groups. The first group only received a standard rabbit diet as the control, the second group (T1) was fed with the same diet as that of the control plus 0.75 ml of fish oil, and the third group (T2) received the same feeding as that of the control plus 1.5 ml of fish oil. Fish oil contained Omega-3 for both treated groups (Fisher Branch, Manitoba, Canada). The diets were prepared by mixing the required amount of standard ratio of the rabbit diet with the appropriate concentration of Omega-3.

2.2. Hematological and Blood Tests

Blood samples were collected at the beginning of the experiment from the marginal ear veins as the zero point, according to the method previously described (2). After 60 days, at the end of the experiment, blood samples were collected again, this time from the heart, and then placed in sterile tubes to separate blood serum for the analysis of biochemical parameters.

2.3. Determination of the Body Weight

The body weights of rabbits were determined at the beginning of the experiment. Afterward, the weights were taken every two weeks. These weights were measured as described by Al-Okaily (13).

2.4. Blood Tests

At the beginning of the experiment, blood was collected from the marginal ear veins of each rabbit. It was fasted for 6 h, and then, the samples were put into different tubes. The first and the second tubes were for hematological and biochemical analyses, respectively. The tubes were centrifuged at 1,000 rpm for 10 min. The blood serum was, then, collected and stored at -80° C. Serum samples were used to determine the levels of urea, glucose, total protein and cholesterol, as well as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes. Furthermore,

chemical blood samples were taken to measure hemoglobin (Hb), white blood cells count (WBCs), packed-cell volume (PCV), and Neutrophil/ Lymphocyte percentages, according to the methodology proposed by El-Moghazy, Zedan (11).

2.5. Statistical Analysis

One-way ANOVA (analysis of variance) was run to compare the control group with the treated groups. Afterward, the post hoc Student-test was conducted. The findings are presented as mean \pm SEM with *P* \leq 0.05 values considered significant. The statistical analysis was performed by the SPSS software (version 2017).

3. Results

3.1. Performance Parameters

Table 1 shows changes in physiological features of the rabbits fed with different levels of fish oil, compared to the control group. The obtained data revealed that the treated groups with different fish oil concentrations (T1=0.75 ml; T2=1.5 ml) showed significant changes in their physiological values. Body weights were measured every two weeks in the experimental groups. There was a gradual increase in the body weight gain of the treated groups, estimated at 1750 ± 200 g in T1 and 1.900 ± 150 g in T2, compared to the body weight of the control group measured at 1.490 ± 150 g.

Daily feed intake increased significantly ($P \le 0.05$) in rabbits fed with a ration supplemented with a high concentration of fish oil (T1), whereas it was lower in (T2), and it recorded a further decrease ($P \le 0.05$) in the control group. Meanwhile, feed conversion ratios were calculated for these groups.

3.2. Biochemical Tests

Biochemical values illustrated significant differences between the treated groups and the control group ($P \le 0.05$). Blood serum samples were tested for urea, glucose, total protein and cholesterol, as well as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes. The effects of the fish oil supplement with different concentrations were significant on these biochemical values ($P \le 0.05$). However, a high concentration of fish oil (Omega-3) was highly significant, compared to a low concentration.

These results are presented in table 2 demonstrating the progressive increase with adding different concentrations of fish oil to the rabbit diet. The urea level (mg/dl) was 37.00 ± 1.00 (mg/dl) in the control and then increased gradually to record 40.00 ± 1.30 in T1, compared to T2, in which it significantly reached (42.50 ± 1.20). The total protein level was (3.60 ± 0.15 mg/dl) in the control while it was (3.65 ± 0.17) in T1 and (3.50 ± 0.16) in T2. The glucose values significantly increased in the control (85.50 ± 5.00 mg/dl), compared to the treated groups with (83.75 ± 4.50 mg/dl) in T1 and (87.00 ± 5.30) in T2. Additionally, the cholesterol levels were measured at (90.60 ± 4.20 mg/dl), (100.30 ± 6.10), and (105.50 ± 8.15) in the control, T1, and T2 groups, respectively.

Table 2 illustrates the levels of AST and ALT serum after eight weeks in the control and treated groups. The level of serum AST significantly increased in T1 (80.50 ± 5.05 IU/L) and T2 (82.00 ± 6.20 IU/L), compared to the control group (75.00 ± 6.15 IU/L). Notable decreases in the levels of ALT were significantly recorded in T1 and T2 at (26.00 ± 2.00 IU/L) and (21.50 ± 1.80 IU/L), respectively. On the other hand, it was recorded at (19.50 ± 1.95 IU/L) in the control group.

3.3. Hematological Tests

Table 3 shows the results of hematological tests on rabbits administrated with different concentrations of fish oil. The levels of PCV (%) were statistically elevated in T2, compared to T1 and the control groups. The Hb level also statistically increased in T2 (951.8%), compared to T1 and the control groups. Conversely, the results of WBCs, Neutrophil (%), and Lymphocyte (%) were significant in T2, compared to T1 and control groups that recorded no significant differences and the values even fluctuated.

Groups	Feed intake (g)	Bodyweight (kg)	FCR
Control	234.40±0.50 ^a	1.590±0.15°	0.130±0.30 ^b
T1 (0.75 mg/ml)	214.37±0.75 ^b	1.750±0.14 ^b	0.140±0.25ª
T2 (1.5 mg/ml)	210.41±0.65°	1.910±0.16 ^a	0.150±0.23ª

Table 1. Performance parameters of rabbits fed different levels of fish oil supplement

*The values within columns indicate mean \pm SEM, and different letters show significant differences at (P<0.05)

Table 2. Biochemical responses of rabbits fed with different concentrations of fish oil supplement in their diets

Groups	Urea (mg/dl)	Total protein (mg/dl)	Glucose (mg/dl)	Cholesterol (mg/dl)	AST (IU/L)	ALT (IU/L)
Control	37.00±1.00 ^b	3.60±0.15 ^b	85.50±5.00 ^b	90.60±4.20 ^b	75.00±6.15 ^b	26.00 ± 2.00^{a}
T1 (0.75 mg/ml)	40.00±1.30 ^{ab}	3.65±0.17 ^b	83.75±4.50°	100.30±6.10 ^{ab}	80.50 ± 5.05^{a}	21.50 ± 1.80^{b}
T2 (1.5 mg/ml)	42.50±1.20 ^a	$3.50\pm0.16^{\rm a}$	87.00±5.30ª	105.50±8.15ª	82.00±6.20 ^a	19.50±1.95 ^b

*Values within columns indicate mean \pm SEM and different letters show significant differences at ($P \le 0.05$).

AST: aspartate aminotransferase

ALT: alanine aminotransferase

Table 3. Hematological parameters of rabbits fed with different concentrations of fish oil supplement in their diet

Groups	Packed cell volume (%)	Hemoglobin (mg/dl)	White Blood cell (103u/l)	Neutrophil (%)	Lymphocyte (%)
Control	39.0±1.15 ^b	8.88 ± 0.80^{b}	5000 ± 250^{b}	38.0±0.50 ^b	55.5±1.00 ^b
T1 (0.75 mg/ml)	40.0 ± 1.17^{b}	9.00±0.75 ^b	5500±260 ^{ab}	40.0 ± 0.45^{b}	57.0±1.05 ^{ab}
T2 (1.5 mg/ml)	46.0 ± 1.16^{a}	10.50±1.05 ^a	5800±265ª	42.0±0.60 ^a	60.0 ± 1.15^{a}

* The values within columns indicate (Mean \pm SEM) and different letters show significant differences at ($P \leq 0.05$).

4. Discussion

The present study aimed to evaluate changes in the performance features of rabbis fed with various levels of fish oil, compared to the control group. The data showed that treated groups with different concentrations of fish oil demonstrated significant values in these tests. Furthermore, there was an increase in the body weight and FCR among treated groups, in comparison with the control group. This improvement has also had significant effects on the cost of animal production considering the high demand for animal meat (1). These results also present progressive enhancement bv adding various concentrations of fish oil to the rabbit diet (3).

There was a significant decrease in feed intakes in rabbits fed with a ration supplemented with both a high concentration of fish oil (T1) and a low level of fish oil (T2), compared to that in the control group. This finding indicated that high-fat diets led to a drop in the feed consumption of treated rabbits (5).

The present results showed that the supplementation of economic rabbit diets with fish oil reduced the concentration of glucose and cholesterol levels. These findings were in line with (5) reporting that adding omega-3 from fish oil reduced the concentrations of plasma cholesterol and glucose level by inhibiting triacylglycerol synthesis in the liver. Unfortunately, the present study did not analyze plasma low-density lipoprotein (LDL) and high-density lipoprotein values, which were found significant in previous studies (14). Omega-3 fatty acids have been reported to induce atherosclerosis without influencing plasma cholesterol levels in hypercholesterolemic cholesterol-fed rabbits and have also been reported to increase the LDL cholesterol levels in cholesterol- and casein-fed rabbits (7, 15).

The use of fish oil in the rabbit diet caused a significant increase in the level of liver enzymes, especially AST and ALT, which are important parameters for a healthy liver in animals (16). The levels of AST and ALT significantly increased and decreased, respectively in the treatment group receiving a high dose of fish oil, compared to the one receiving a low level of fish oil supplement and the control group. These findings were consistent with those demonstrated by El-Moghazy, Zedan (11).

Hematological trials revealed that PCV significantly increased in rabbits given various amounts of fish oil. However, it was significantly higher in the treated group given a high dose of fish oil, compared to the treated group given a low dose of fish oil and the control group. Furthermore, the Hb level increased in animals given a high dose of fish oil, compared to those receiving a low dose and the control group. These findings were consistent with the findings of El-Moghazy, Zedan (11).

Similarly, regarding WBC, Neutrophil (%), and Lymphocyte (%), significant values were observed in rabbits treated with a high concentration of fish oil, in comparison with those receiving a low concentration and the control group. There were no significant differences between these groups in terms of results, and the values fluctuated. However, there was an abnormally high Neutrophil level in differential WBC counts, which is associated with bacterial illness, but greater Lymphocyte counts are associated with a viral illness. These findings were in line with those of Cartuche, Pascual (5), as well as Hutanu, Horvath (17).

Based on the findings of this study, it was determined that oral administration of fish oil daily at 0.75 mg/ml or 1.5 mg/ml to rabbits 60 days before conception can improve performance trials and cause significant improvements in biochemical and hematological results. However, compared to high doses used on rabbits, these changes have a minor impact on these characteristics.

Authors' Contribution

Study concept and design: M. M. D.
Acquisition of data: H. T. K.
Analysis and interpretation of data: A. M. K.
Drafting of the manuscript: M. M. D.
Critical revision of the manuscript for important intellectual content: H. T. K.
Statistical analysis: A. M. K.
Administrative, technical, and material support: M. M. D.
D.

Ethics

All procedures in this study were approved by the ethics committee of the University of Baghdad, Baghdad, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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