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Effect of Tallow and Olive Oil on Some Biochemical and Hematological Parameters in Healthy Male Albino Rats

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Abstract

Fats, a broad term for fats and oils, are sourced from either plants or animals. They function as energy storage and are crucial in various biological processes. This study aimed to examine the effects of tallow and olive oil on certain hematological and biochemical parameters in healthy albino rats. Twenty-eight male albino rats were divided into four groups, each containing seven animals. Groups given different treatments orally: Distilled water (control group), sheep tallow (Sh-T group), bovine tallow (B-T group), and olive oil (OO group). At the end of the third month, blood samples were collected from the fasting rats for hematological and biochemical analysis. Our results show that the oral administration of Sh-T, B-T, and OO did not cause significant changes in body weight (BW), food intake (FI) and level of serum of fibrinogen (FIB), albumin (ALB), C-reactive protein (CRP), or blood cell counts, including white blood cells (WBCs), red blood cells (RBCs), lymphocytes, neutrophils, platelets, and the erythrocyte sedimentation rate (ESR), when compared to the control group. However, total protein levels in the Sh-T and B-T groups, as well as globulin levels in the Sh-T and OO groups, showed significant increases, whereas the OO and B-T groups demonstrated a significant decrease in monocyte and eosinophil counts compared to the control group. It can be concluded that neither sheep nor bovine tallow nor olive oil negatively affects body weight or serum liver proteins, nor do they induce substantial hematological alterations.

Article Info

Keywords:
Tallow, Olive Oil, Albino Rats, C-reactive protein, Fibrinogen, Sheep, Bovine

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1. INTRODUCTION

Lipids are a heterogeneous group of compounds insoluble in water but miscible in organic solvents [1], [2] and represent the second major source of energy after carbohydrates [3], [4]. Fats are a type of simple lipid known as a general term for fats and oils, which have the same composition and differ only physically since fat is a solid while oil is a liquid at room temperature [5]. Lipids are commonly distributed through the plant's parts (seeds, fruits, and nuts) and animal bodies (adipose tissues, bone marrow, and nervous tissues) [1].

Lipids play an essential role in nearly all aspects of biological life and are a main component in the cell structure [5]. Furthermore, a diet based on good-quality fats is beneficial for the appropriate functioning of the body, including the nervous and cardiovascular systems and the hormonal balance [6] . The characteristics of fats are determined by the fatty acids they contain; saturated fatty acids (SFAs) have no double bonds within the fatty acid chain, monounsaturated fatty acids (MUSFAs) have a single double bond, while polyunsaturated fatty acids (PUSFAs) contain two or more double bonds [7].

Tallow is obtained from rendering animal suet, forming the hard and white fatty layers surrounding animals' organs. It is a valuable source of concentrated energy and essential fatty acids necessary for growth and devel-

opment [8].

Olive oil is the main fat component of the Mediterranean diet [9], which is known for its significant antihyperlipidemic potential [10] and low risk for cardiovascular disease [11]. People who consume a lot of olive oil may be protected from developing many diseases, such as cardiovascular disorders, type II diabetes, obesity, metabolic syndrome, and other illnesses [12].

Hematological parameters are linked to various health indices and play a significant role in the routine clinical assessment of health [13]. Additionally, these parameters are important for diagnosing and evaluating the severity and progression of certain diseases, such as hyperlipidemia [14]. Moreover, many of these parameters are strongly associated with cardiovascular disease[15] . They provide valuable information regarding inflammation, tissue necrosis, the presence of stress factors, and infections in visceral organs [16], [17], [18].

The liver maintains homeostasis in the living system as it is involved in biochemical pathways necessary for growth and fighting against diseases [19]. It affects all major systems' functions due to its tight relationship to lipid metabolism [20]. It is believed that the liver is extensively affected by dyslipidemia, and the alterations of the lipid level may impact liver metabolism and damage the hepatocyte tissue [21], [22], [23]. Also, excessive accumulation of lipids within hepatocytes due to a lack of equilibrium between lipid formation and lipid decomposition and excretion leads to fatty liver and hepatic steatosis [24], [25] and, in sequence, leads to hyperlipidemia [26]. The total protein, bilirubin, and albumin concentrations may indicate the state of the liver and the type of damage [27]. Additionally, normal levels of globulins and fibrinogen in the serum indicate a healthy liver [26]. To better clarify the effect of naturally sourced fats on health, the present study aimed to investigate and compare the effects of tallow, from sheep and bovine, and olive oil on several important biochemical and hematological parameters in healthy rats, as little is known about this topic.

2. MATERIALS AND METHODS

2.1. Tallow preparation

The fat samples of sheep and bovines were taken from local animals in Thamar city, up to 12 months of age, at the official abattoirs. Animals' body fats were collected from the mesentery and areas surrounding the kidney, rumen, and heart, as well as from the sheep's tail. For tallow preparation, fat samples were kept in a deep freezer at $-18\,^{\circ}\mathrm{C}$ for 24 hours and then cut into small pieces and placed in a large pan on a cooker with low heat. The melted tallow was poured off on layers of gauze for filtration and to remove any additional tissues. Ready-made and refined tallow was poured and kept in glass mason jars.

2.2. Olive oil:

It was in virgin liquid form and purchased from a commercial store in Thamar City, Yemen.

2.3. Experimental Animals

Twenty-eight healthy adult Wistar albino male rats (Rattus rattus), 6 months old and weighing between 256 and 266 g, were obtained from the Animal House of the Faculty of Science at Sana'a University. The rats were kept in a suitable room for the experiment at a temperature of 22±2 ℃ in clean stainless-steel cages, with a 12-hour light and 12-hour dark cycle, and were provided with a sufficient diet and water *ad libitum*. They were maintained for conditioning for one week and then randomly grouped. The animals received a nutritional diet prepared according to the [28] diet formula. The "Committee of Experimental Animals Care and Use" approved the study protocol at the Biological Science, Faculty of Science, Sana'a University, Yemen (Ethical code: BAHSS 101), and all experimental procedures adhered to the guidelines.

2.4. Experiment design

The present experiment was conducted for three months on albino rats, which were randomly divided into four groups and treated daily as follows:

- Group I (C) served as control and was orally administered 1 mL of DW.

- Group II (Sh-T) was orally administered 1ml /kg b. w./day of sheep's fat.

- Group III (B-T) was orally administered 1ml /kg b. w/day of bovine fat.

- Group IV (OO) was orally administered 1ml /kg b. w/day, daily, of olive oil.

2.5. Biological evaluation

The food intake was recorded daily during the experimental period, while body weight (BW) was taken every 15 days.

Feed intake (FI) was calculated by the equation described by [29] as follows: Feed intake = Initial weight of diet (g) - weight of diet lost (g).

Body weight gain (BWG, g) was calculated by the formula described by [30] as follows:

BWG (g) = final weight (g)-initial weight (g)

The percentage change in body weight is calculated using the formula: [(Final Weight - Initial Weight) / Initial Weight] x 100.

2.6. Blood Sample Collection

At the end of the experiment, blood samples were collected from the rats after fasting overnight from the orbital sinus using a capillary tube that pressed behind the jaw angle [31], as shown in Figure 1. Each blood sample was divided into three parts: The first part was allowed to clot at room temperature for 4 h and then centrifuged at 2000 rpm for 15 minutes. Isolated serum was stored at $-20\,^{\circ}C$ until analysis and used for biochemical analysis of liver protein. The second part was placed in test tubes containing 0.3 ml of trisodium citrate, as an anticoagulant, in a ratio of 9:1. The blood was mixed and spun within 2 h, and the platelet-poor plasma was separated for use in fibrinogen assay [32]. The third part was placed in test tubes containing heparin (5000 units/ml) as an anticoagulant, these blood samples were processed for hematological indices in less than three hours.

Figure 1. Collecting blood samples from the orbital sinus

2.7. Experimental Tests

2.7.1. Determination of liver protein levels

- **2.7.1.1.**Total protein (TP) content was determined via the biuret reaction described by [33] and [34]. The principle of this reaction is that serum proteins react with copper (Cu^{2+}) in an alkaline solution to form a violet biuret complex that was developed relative to the number of peptide bonds in the determined protein and measured at 550 nm.

- **2.7.1.2.** Albumin level (ALB) was determined by a dye-binding technique that utilizes albumin's ability to form a stable complex with bromocresol green dye [35]. It was measured at 628 nm.

- **2.7.1.3.** Globulin level was calculated from the difference between total protein and albumin [36].

- **2.7.1.4.** Plasma fibrinogen (FIB) was estimated according to [37] and [38]. This method was based on the fact that when an excess of thrombin is present, the fibrinogen is converted into fibrin fibers, subsequently forming a detectable clot. In this test, the diluted plasma sample was mixed with a thrombin reagent, and the timing of the initial clot formation was recorded. The FIB concentration was obtained from a reference curve calibrated with human plasma fibrinogen and reported in milligrams per deciliter (mg/dL). Automated colorimetric analysis methods were employed in the biochemical analysis of TP and ALB, with the assay kits for TP, ALB, and FIB sourced from Biolabo co/French. FIB results were read using the semi-automated Coag. 2D from Diagon Co, Hungary, while TP and ALB results were analyzed using the Biochemistry Analyzer RX50V instrument, and the standard clinical equations for each parameter were calculated. Measurements were conducted at Al-Mageed Medical Lab, Sana'a.

- **2.7.1.5.** C-reactive protein (CRP) was measured via the nephelometry immunoassay method using commercial kits (Hipro Biotechnology Co., Germany) in Al-Mageed Medical Lab, Sana'a.

2.7.2. Hematological parameter determination

Total White Blood Cell (WBCs) Count, Differential Count of WBCs, Red Blood Cell (RBC) Count, and Platelets (PLts) Count were measured by an automated hematology analyzer (Dymind DH36, Co. China). The erythrocyte sedimentation ratio (ESR) was measured by the Westergren tube method [39]. All tests were conducted in Al-Mageed Medical Lab in Sana'a.

2.7.3. Statistical analysis

All presented data were expressed as a mean ± SD. Statistical significances between groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey Multiple. Comparisons were made as a post-test using the computer prism program (8.0.2.263) (San Diego, CA, USA). Differences between and among the groups were considered significant at $P < 0.05$ or less.

3. RESULTS

3.1. Body Weight and Food Intake

The results presented in Table 1 showed no significant differences in body weight gain (BWG) and food intake (FI) between the control group and the fat treatment groups, tallow or olive oil. Furthermore, concerning the percentage change in body weight, the sheep group had the closest value to the control (31.91% and 31.83%, respectively), while the bovine group had the farthest value (25.23%).

Table 1. Rats' body weights and food intake during the experiment

Biological Evaluation / Groups	Initial body wt, Mean± SD	Final body wt, Mean± SD	Body weight gain (BWG), Mean± SD	Percentage of body weight change%	Food intake (FI) Mean± SD
C	262.9± 8.95	346.6± 13.19	83.71± 10.26	31.83 %	18.33 ± 1.86
Sh-T	261.0± 16.21	344.3± 18.66	83.29± 10.84	31.91 %	18.28 ± 4.94
B-T	262.4± 15.99	328.6± 45.72	77.57± 9.88	25.23 %	18.17 ± 1.21
OO	261.3± 7.29	340.9± 6.25	79.57± 5.60	30.46%	18.07 ± 1.22

Results expressed as Mean ± SD for 7 rats in each group. C: control, Sh-T: sheep's tallow, B-T: bovine's tallow, OO: Olive oil

3.2. Liver Proteins Level

Data from Figure2-A showed that the total protein levels were significantly increased in the Sh-T and B-T groups compared to the control group ($P < 0.05$), as the increase in the OO group did not reach significance compared to the control group. In contrast, albumin levels of the plasma showed no significant differences among the three treatment groups (Sh-T, B-T, and OO) compared to the control group, nor when comparing the groups to each other, as illustrated in Figure2-B.

Figure2- C showed a significant increase in globulin level in the Sh-T and OO groups compared to the control group ($P < 0.01$, $P < 0.05$, respectively), whereas a non-significant change was found in the B-T compared to the control group.

Regarding FIB level in serum, a non-significant change was observed in this level in the three treatment groups (Sh-T, B-T, and OO) compared to the control group, while a significant decrease was observed in the OO group compared to the Sh-T and B-T groups ($P < 0.01$, $P < 0.001$, respectively) as shown in Figure2-D.

No significant change was observed in CRP levels when comparing the control group with the three treated groups (Sh-T, B-T, and OO), nor when comparing the treated groups with each other. The highest CRP value was found in the Sh group (see Figure 2-E).

3.3. Hematological Results

As shown in Table 2, most parameters of blood elements in lipid-treated groups were very close to the control group, in general terms. However, lipid treatment results in slight, non-significant increases in total WBC and RBC counts, as well as lymphocyte and neutrophil numbers. Conversely, monocyte and eosinophil numbers decreased significantly with B-T and OO treatments. The same treatments also induced non-significant decreases in platelet number and non-significant increases in ESR. It is worth noting that blood parameter levels in the Sh-T group were closest to the control group.

4. DISCUSSION

Our results of the effect of Sh-T, B-T, and OO on the BWG and FI of rats show that non-significant changes occurred between the control and the treated groups. Besides, compared to the control group, the largest difference was observed in the B-T group, while the smallest difference was found in the Sh-T group. In a similar topic, Matsuo *et al.* [40] studied the impact of different dietary fats on body fat in rats and found much the same weight gain in each group, but they found that beef tallow led to more body fat than soybean or sunflower oils. Also, Haggag *et al.* [41] investigated the effects of feeding diets containing different dietary fats and oils, one of them OO, on the BWG and FI of rats; they indicated that no significant differences in FI between groups. Furthermore, Abdullah *et al.* [42] showed that the OO administration decreased FI but non significantly, while Khaliq *et al.* [43] showed a significant decrease in FI with the OO administration. The liver maintains homeostasis in the living system. It is involved in biochemical pathways necessary for growth and fighting against diseases [19], so this study focuses on the investigation of animal fat, represented by sheep fat and bovine fat, as well as olive oil, on the health status of the liver through an assessment of the level of liver proteins, which reflects the efficiency of the liver in performing its function. Our results indicate no significant decrease in serum liver protein levels (TP, ALB, Globulin, FIB, or CRP) after treatment with tallow or olive oil, which reflects the safety of tallow and olive oil on liver health. Moreover, in the present study, levels

Table 2. The hematological indicators in the experimental groups

Groups / Hematological indicators	C	Sh-T	B- T	OO
WBCs ($10 \wedge 3/\mu$L), mean± SD	9.886 ± 2.27	10.0 6±1.165	10.21± 0.612	9.419 ±1.068
RBCs × $10 \wedge 6(mm3)$, mean± SD	8.743±0.403	9.029±0.518	8.943±0.276	8.703 ±0.241
Lymphocyte (%), mean± SD	47.14±2.610	49.43±2.370	48.29±5.648	49.00±6.028
Monocyte (%), mean± SD	6.857±1.069	5.714±0.755	5.429±0.786 a: *	5.000±0.577 a: **
Neutrophils (%), mean± SD	40.86±3.132	42.43±3.155	43.29 ±5.851	42.57±5.593
Eosinophils (%), mean± SD	4.286±0.951	3.286±0.488	3.000±0.577 a: *	2.143±0.899 a: ***, b: *
Platelets $10 \wedge 3$ (cell/ μL), mean± SD	407.1±7.904	416.3 ±37.48	369.1 ±48.85	386.0 ±45.39
ESR. (mm/1h), mean± SD	6.143 ±1.069	6.714 ±1.496	7.143±1.464	7.286±0.755

Results expressed as mean ± SD for 7 rats in each group. **a**: significant compared with the control group, **b:** significant compared with the Sh-T group. *= P < 0.05, **=P < 0.01, and ***=P < 0.001.
C: control, **Sh-T**: sheep's tallow, **B-T:** bovine's tallow, **OO:** Olive oil.

of TP and Globulin were increased significantly under the44tietz1976fundamentals effect of tallow and olive oil. Besides, ALB, a major protein that circulates in the bloodstream and is synthesized in the liver [44], showed levels in all treated groups within the natural range. In the present study, most blood parameters showed nonsignificant changes under the effect of fat treatment, except monocyte and eosinophil

numbers, which decreased significantly with B-T and OO treatments. Otherwise, slight changes in blood parameters were observed in B-T and OO groups, while the values in the Sh-T group were very close to the control. Our WBC results align with Abdullah *et a*l. [42], who observed non-significant change of WBC in their study of the effect of animal fat and OO on hematological parameters. Also, they did not find a significant change in RBCs by OO treatment, but a significant increase was observed by animal fat, all compared to the control.

Furthermore, the major indicators of inflammation, which appear to be positively correlated with cardiovascular disease and atherosclerosis, such as ESR, Platelets, WBC [45] and [46], fibrinogen, and CRP [47], [48], in the current study were at the normal levels that do not pose any threat to the possibility of progression of atherosclerosis. However, the decrease in fibrinogen obtained in the OO group compared to the B-T and Sh-T groups may be attributed to the properties of the fatty acids in its composition. Moreover, [49][and [50] demonstrated that olive oil and its different phenolic contents decreased fibrinogen levels. Also, it has been previously proven that olive oil has properties that reduce fibrinogen levels [51].

5. CONCLUSION

This study demonstrates that sheep tallow, bovine tallow, and olive oil are safe for consumption by healthy animals. Our conclusion

is supported by their normal and non-negative effects on body weight, serum liver protein levels, and hematological parameters. Notably, the results observed by sheep tallow treatment were the closest to the normal values.

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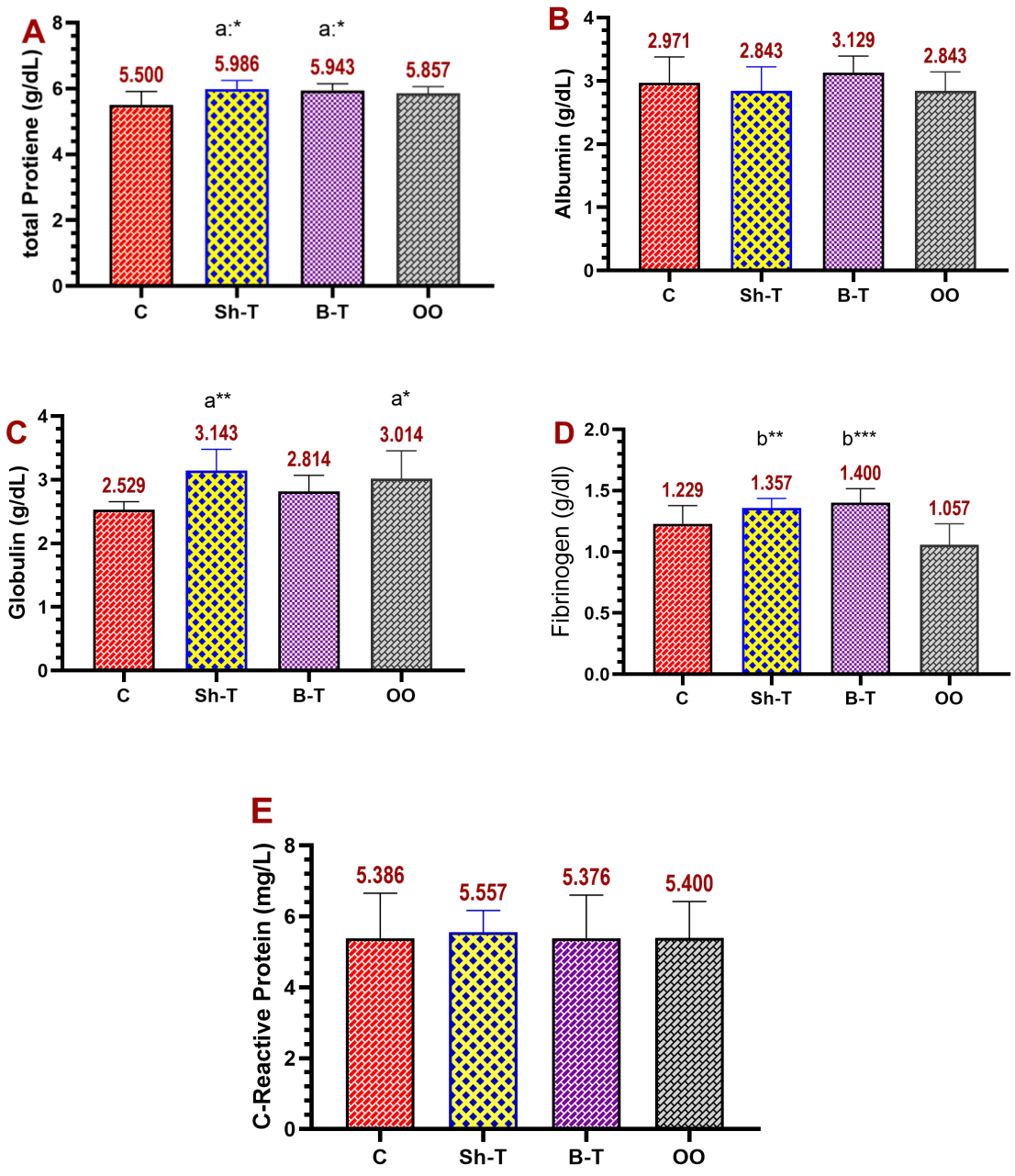

Figure 2. Liver Proteins levels in serum of the experiment groups, A: Total protein level, B: Albumin levels, C: Globulin level, D: Fibrinogen, E: C-Reactive protein. Results expressed as Mean ± SD for 7 rats in each group. *= P < 0.05, **=P < 0.01, and***=P < 0.001. C: control, Sh-T: sheep's tallow, B-T: bovine's tallow, OO: Olive oil. a: significant compared with the control group, b: significant compared with the OO group.

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