International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 12S, 2025
https://www.theaspd.com/ijes.php

Effect of Adding Extracted Conjugated Linoleic Acid (CLA) on Hypercholesterolemic Female White Rats and Its Comparison with Commercial Conjugated Linoleic Acid

Nada Gamal Azez[1], Athraa Harjan Mohsen[2]
[1] **Department of Food Science, Faculty of Agriculture, University of Kufa, Al-Najaf, Iraq Email:** nadaj.alduhaidahawi@student.uokufa.edu.iq
[2] **Department of Food Science, Faculty of Agriculture, University of Kufa, Al-Najaf, Iraq Email:** athraa.alduhaidahawi@uokufa.edu.iq

Abstract

The results of dosing and the effect of different concentrations of conjugated linoleic acid (CLA) on hypercholesterolemic female white rats fed a high-fat diet indicated a significant decrease in cholesterol level to **78.46 mg/dl** at **2% CLA concentration**, compared to the group fed a high-fat diet without CLA, which showed an elevated cholesterol level of **119.67 mg/dl**. Triglyceride (TG) levels decreased across all concentrations compared to the high-fat treatments, reaching 78.88 mg/dl at the 2% concentration, while they were 81.82 mg/dl and 94.80 mg/dl at the 0.5% and 1% concentrations, respectively. In the control group, the level was 32.1 mg/dl. Administration of conjugated linoleic acid (CLA) at varying concentrations resulted in notable effects on HDL and LDL cholesterol levels in laboratory animals. HDL concentrations increased across all treated groups, with the highest level observed at the 2% concentration (45.36 mg/dl), compared to 42.70 mg/dl and 41.40 mg/dl at the 1% and 0.5% concentrations, respectively. In contrast, the control group recorded a lower HDL value of 32.1 mg/dl. LDL levels, on the other hand, decreased significantly at the 2% concentration, reaching 44.10 mg/dl, compared to higher levels at the lower concentrations. Furthermore, the hepatic enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) showed reduced activity at the 2% CLA concentration, with values of 40.98 IU/L and 55.18 IU/L, respectively.

1. INTRODUCTION

The recent emergence of the conjugated linoleic acid (CLA) market and the growing consumer interest in its health benefits have accelerated research efforts into its natural sources (Zongo et al., 2021).

CLA is an essential polyunsaturated fatty acid primarily found in foods derived from ruminant animals, such as milk and meat, making their consumption a convenient way to enhance the bioavailability of CLA in the human body (Duchemin et al., 2013). Conjugated linoleic acid (CLA) refers to a group of positional and geometric isomers of linoleic acid (LA, 18:2 n-6) that contain conjugated double bonds. This fatty acid occurs naturally in food and includes several isomeric forms, among which **cis-9, trans-11** and **trans-10, cis-12** are the most common and biologically significant isomers found in nature (Han et al., 2002; Basak & Duttaroy, 2020).

These two biologically active isomers have been associated with a reduction in body fat accumulation, inhibition of tumor formation, and enhancement of the immune system in animal studies. They

have also been reported to improve the structure and function of high-density lipoprotein (HDL) cholesterol, playing a potential role in cardiovascular disease prevention (Vaisar et al., 2022).

Cholesterol is a waxy, fat-like substance found in all cells of the body. It is essential for the production of hormones, vitamin D, and substances that aid in the digestion of food. The body produces all the cholesterol it needs; however, cholesterol is also present in animal-based foods such as egg yolks, meat, and cheese (Khodadadi et al., 2020).

2: MATERIALS AND METHODS

1-2: Experiment Animals

This study was conducted at the Sigma Laboratory in Al-Najaf Al-Ashraf under the supervision of specialized professors, following the approval of the Bioethics Committee as per letter No. (2116), Appendix No. (1), and in accordance with the experimental protocol for animal care and the guidelines of the Animal Care Council.

A total of 36 female Albino rats of the Sprague Dawley strain, aged 60 days and weighing between 180–200 grams, were used in this experiment. The rats were housed in special breeding cages in a clean, environmentally controlled room maintained at a temperature of (20 ± 2) °C for 24 hours a day. They were provided with water and a standard pellet diet for two weeks prior to the experiment to allow for acclimatization.

The rats were then randomly divided into six groups, each consisting of six rats. Each group was considered a single replicate. The rats' weights were recorded before the start of the experiment and at its conclusion, and the weight differences were calculated accordingly.

1-1-2: Diet

The high-fat diet was based on the formulation provided by Levin and Dunn-Meynell, as described by Levin & Dunn-Meynell (2002). This high-fat diet included all the nutritional components listed in Table (3-5). The high-fat pellets were used to feed the rats, inducing hypercholesterolemia over a 4-week period (Kadir Abdul et al., 2015).

Table (2-1) shows the components of the high-fat diet for laboratory animals.

High-Fat Diet		**Standard Diet**	
Nutrient Components	%100/g	%100/g	
Carbohydrates	43	48.8	
Protein	17	21	
Fat	40	3	
High-Fat Diet 100 g/g		**Standard Diet** 100 g/g	
Powdered rat chow	68.0	Calcium	0.8
Oil	6.0	Phosphorus	0.4
Margarine	6.0	Fiber	5
Dried milk powder	20.0	Moisture content	13
-	-	Ash	8
Total Energy (kcal/100 g)	414.0	**Total Energy (kcal/100 g)**	306.2

2-1-2: Experiment Design

A total of 36 female white rats, aged 60 days, were used in the experiment. They were randomly divided into six groups, with each group consisting of six rats. The animals were fed a high-fat diet for 28 days and treated as follows:

- **Group 1 (Control Group):** Fed a standard (normal) diet.
- **Group 2 (High-Cholesterol Control Group):** Fed a high-cholesterol diet.
- **Group 3 (Commercial Group):** Administered standard linoleic acid.
- **Group 4:** Administered a high-cholesterol diet along with extracted linoleic acid at a dose of 0.5 µg/kg.
- **Group 5:** Administered a high-cholesterol diet along with extracted linoleic acid at a dose of 1 µg/kg.
- **Group 6:** Administered a high-cholesterol diet along with extracted linoleic acid at a dose of 2 µg/kg.

After 28 days of treatment with both the extracted and commercial conjugated linoleic acid, the animals were sacrificed humanely using chloroform anesthesia. Blood samples (1–2 mL) were collected via cardiac puncture using gel tubes that promote clotting and serum separation. The serum was then used to assess blood biochemical parameters following the administration of both forms of linoleic acid.

2-2: Blood Samples Collection

To alleviate pain, the rats were injected intramuscularly with a mixture of ketamine (5 mg/100 g) and xylazine (0.25 ml/100 g). Using 10 ml medical syringes, venous blood samples were collected from all experimental groups. For the measurement of physiological parameters, part of the blood samples was transferred into tubes containing anticoagulant agents to prevent clotting. The other portion of the samples was placed into test tubes without anticoagulants and left at room temperature for 10–15 minutes. The samples were then centrifuged at 3000 rpm to separate the serum from the other blood components. The serum was collected and placed in tubes for subsequent biochemical analyses. The biochemical and physiological tests were performed as described by (Van 1977).

2-3: Biochemical Parameters Measurement

2-3-1:Estimation of Total serum cholesterol

The enzymatic method was used to estimate total serum cholesterol according to the method of (Siedel et al. 1981).

Absorbance was measured at a wavelength of 500 nm according to the following equations:

$$\text{Cholesterol Ester} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol free} + \text{Fatty acid}$$

$$\text{Cholesterol Ester} + O2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholest-4-en-3+H2O2}$$

Peroxidase

$$2H_2O_2 + \text{Phenol} + \text{4-aminoantipyrine} \longrightarrow \text{quinoneimine} + 4H_2O$$

The analysis was performed according to the following steps:

2.5 mL of the working solution was placed into three test tubes. The working solution consisted of:

1. **a. Buffer solution:** Sodium cholate at a concentration of 3.74 mL, phosphate buffer at a concentration of 0.1 mmol/L, and phenol at a concentration of 15 mmol/L.

b. Enzyme reagent: Containing peroxide at a concentration of 1000 international units per liter (IU/L) and aminoantipyrine at a concentration of 0.5 mmol/L.

2. The cholesterol standard solution with a concentration of 5.17 mmol/L, equivalent to 200 mg/L, was prepared. Then, 0.025 mL of the sample serum was added to the first test tube, 0.025 mL of the cholesterol standard solution to the second test tube, and 0.025 mL of distilled water to the third test tube. The contents of each tube were thoroughly mixed, and the three tubes were then incubated in a water bath at 37°C for 5 minutes. The spectrophotometer was initially calibrated using distilled water and then with the reagent blank at a wavelength of 500 nm. The absorbance of the standard solution and the sample were read. The cholesterol concentration in the serum sample was calculated using the following equation:

$$\text{Total S. Cholesterol (ml/dl)} = \frac{\textbf{A Sample}}{\textbf{A Standard}} \times 200$$

2-3-2: Estimation of Serum Triglycerides TG

The enzymatic method described by (Fossati and Prencipe 1982) was used, and absorbance was measured at a wavelength of 505 nm.

2.5 mL of the working solution was placed into three test tubes. The working solution consisted of:

1. a) Buffer solution, composed of para-chlorophenol at a concentration of 2.7 mmol/L, magnesium at 4 mmol/L, and Tris buffer at 100 mmol/L.

b) Enzyme reagent, containing lipase enzyme at 1000 international units per liter (IU/L), glycerokinase enzyme at 200 IU/L, peroxidase at 200 IU/L, and aminoantipyrine at 0.4 mmol/L. The working solution was prepared by mixing the enzyme reagent components with 25 mL of the buffer solution.

2. The triglyceride standard solution with a concentration of 2.29 mmol/L, equivalent to 200 mg/100 mL. Then, 0.025 mL of the serum sample was added to the first test tube, 0.025 mL of the triglyceride standard solution to the second test tube, and 0.025 mL of distilled water to the third test tube. The contents of each tube were mixed thoroughly. The three tubes were incubated in a water bath at 37°C for 5 minutes. The spectrophotometer was calibrated first with distilled water, then with a reagent blank, at a wavelength of 505 nm. Absorbance readings were taken for the standard solution and the sample. The triglyceride concentration in the serum sample was calculated using the following equation:

$$\textbf{Triglycerides Conce (mg/dl)} = \frac{\textbf{A Sample}}{\textbf{A Standard}} \times \mathbf{100}$$

2-3-3: Calculation of low density lipoproteins LDL

The calculation of low-density lipoprotein (LDL) levels was performed using the formula described by (Wilson ,1998).

LDL . Cholesterol (mg/DI) = Total cholesterol - (VLDL +HDL)

3-3-2: Estimation of high density lipoproteins HDL

The method described by (Finley et al. 1978) was used to precipitate lipoproteins present in high-density lipoproteins (HDL) in serum, including LDL and chylomicrons, using phosphotungstic acid in the presence of magnesium ions. The solutions consisted of:

1. **HDL–Cholesterol reagent:** composed of cholesterol esters at a concentration of 1.30 mmol/L.
2. **Precipitating reagent:** composed of magnesium chloride hexahydrate at a concentration of 100 g/L and phosphotungstic acid at 40 g/L.

0.5 mL of serum was mixed with 0.05 mL of the precipitating reagent in a test tube and left to stand for 10 minutes. The mixture was then centrifuged at 3000 rpm for 15 minutes.

Three test tubes were prepared, each containing 3 mL of cholesterol solution. To the first tube, 0.15 mL of the supernatant was added; to the second tube, 0.15 mL of distilled water; and to the third tube, 0.15 mL of the HDL–cholesterol reagent. Each tube was mixed thoroughly.

The three tubes were then incubated in a water bath at 37°C for 5 minutes. The spectrophotometer was calibrated first with distilled water, then with a reagent blank, at a wavelength of 500 nm. Absorbance readings were taken for the standard solution and the sample. The HDL concentration in the serum sample was calculated using the following equation:

$$\text{HDL. Conce (mg/dl)} = \frac{\text{A Sample}}{\text{A Standard}} \times 100$$

3-3-4:Estimation of Liver Enzyme Activity in Serum (ALT, AST)

The method of (Reitman and Frankel ,1975) was adopted to estimate the amount of pyruvate and oxaloacetate released through their reaction with 2,4-dinitrophenylhydrazine (DNPH). The solutions used were those provided in the kit specifically designed for the determination of liver enzyme activities, which was prepared by **BioMérieux Rsa**. The kit includes the following solutions:

1. Buffer solution, consisting of sodium merthiolate at a concentration of 0.1 g/mL, and disodium phenyl phosphate at a concentration of 50 mM (pH 10).
2. Tetramethylbenzidine (TMB) solution, containing 60 mM TMB and sodium arsenite at 75 g/L.
3. Phenol solution 20 King units.
4. Color reagent, consisting of potassium ferricyanide at a concentration of 150 mM.

Table (2-2): Contents of the tubes for the determination of the activity of the two aminotransferase enzymes

Solutions	Sample Tube	Blank Tube
Serum sample	0.1	-
AST/ALT buffer solution	0.5	0.5

Tubes were mixed and incubated at 37°C for 30 minutes		
Serum sample	-	0.5
Dinitrophenylhydrazine (DNPH) solution	0.5	0.5
Tubes were mixed and incubated at room temperature (20–25°C)		
Sodium hydroxide solution	0.5	0.5

The contents of the tubes were thoroughly mixed, and the absorbance was measured at a wavelength of 540 nm. A standard curve was obtained for the estimation of pyruvate, using various concentrations as specified in the kit instructions. The relationship between absorbance and enzyme activity (expressed in U/L) was plotted. One unit of enzyme activity (1 U) is defined as the amount of enzyme that catalyzes the release of one micromole of pyruvate per minute under the specified reaction conditions.

3. RESULTS AND DISCUSSION

3- 1: Effect of extracted conjugated linoleic acid (CLA) and commercial linoleic acid on body weight gain rates of female white rats during the feeding period

The results presented in Table (3-1) indicate significant differences in body weights of the animals treated with various concentrations of extracted and commercial conjugated linoleic acid (CLA) during different feeding periods.(Mohsen, 2019)

Specifically, administration of 2% extracted CLA resulted in a reduction in body weight, with values reaching 230, 227, 224, and 218 g/kg during the first, second, third, and fourth weeks of the feeding period, respectively, compared to the initial body weight of 236 g/kg before feeding on the high-fat diet. In contrast, the body weights of animals administered 1% and 0.5% extracted CLA while on a high-fat diet were 236 and 240 g/kg, respectively.

Further, when administered with the same concentrations of extracted CLA, body weights gradually decreased to 237, 234, 232, and 230 g/kg and to 235, 230, 228, 226, and 224 g/kg, respectively.

On the other hand, commercial CLA also contributed to a reduction in body weight, with values decreasing to 248, 247, 246, and 245 g/kg in the first, second, third, and fourth weeks, respectively, compared to the initial weight of 250 g/kg prior to treatment. In contrast, animals that were not administered any form of CLA showed an increase in body weight from 275 g/kg to 290 g/kg by the fourth week. These findings suggest that conjugated linoleic acid has a weight-reducing effect.(Al-Sallami,2017)

The reduction in the body weights of laboratory animals treated with different concentrations of CLA may be attributed to its physiological effects, such as increased activity of **hormone-sensitive lipase** and decreased activity of **lipoprotein lipase**. In addition, inhibition of adipose tissue development may be due to reduced secretion of **tumor necrosis factor (TNF)** and **leptin hormone** (Park et al., 1997).

Table (3-1) shows the body weights of female rats before and after administration of a high-cholesterol diet.

Treatment Groups	Weight Before Administration	Weight After Administration Week 1	Week 2	Week 3	Week 4

Rats without administration	**257e**	**280e**	**285e**	**288f**	**290e**
Control	**190a**	**188a**	**190a**	**190a**	**188a**
Commercial CLA	**250d**	**248d**	**247d**	**246e**	**245d**
CLA 0.5%	**235b**	**230b**	**228b**	**226c**	**224c**
CLA 1%	**240c**	**237c**	**234c**	**229.7d**	**227.7c**
CLA 2%	**236b**	**230b**	**227b**	**233b**	**217b**

Different letters (a, b, c, etc.) indicate statistically significant differences between groups at $P < 0.05$ based on LSD analysis.

3.2: Biochemical Parameters

3.2.1: Effect of Different Concentrations of Extracted Conjugated Linoleic Acid on Total Cholesterol (TC) and Triglycerides (TG) Compared to Commercial Linoleic Acid

The results of using extracted and commercial conjugated linoleic acid (CLA) and their effects on cholesterol levels are presented in **Table (3-2)**. The data indicate statistically significant differences between treatments in reducing total cholesterol levels. The 2% concentration of extracted CLA resulted in a cholesterol reduction to **78.46 mg/dL**, compared to **119.67 mg/dL** in the hypercholesterolemic control group. Lower concentrations (0.5% and 1%) also reduced cholesterol levels to **82.18 mg/dL** and **98.91 mg/dL**, respectively.

There were also significant differences in cholesterol reduction when using 1% of the commercial CLA, where cholesterol decreased to **92.43 mg/dL** compared to the untreated hypercholesterolemic group.

Significant reductions in **triglyceride (TG)** levels were also observed across all treatments compared to the hypercholesterolemic female rats. The 2% concentration of extracted CLA was the most effective, reducing triglyceride levels to **78.88 mg/dL**, while the 0.5% and 1% concentrations lowered them to **81.82 mg/dL** and **94.80 mg/dL**, respectively. In the hypercholesterolemic group, triglyceride levels were **127.10 mg/dL**. Additionally, the use of commercial CLA led to a reduction in triglyceride levels to **118.60 mg/dL** compared to the untreated group.(Mohsen , 2019)

The decrease in cholesterol markers in the CLA-treated hyperlipidemic rats may be due to the transport of CLA through the plasma via **lipoproteins**, where a portion is absorbed in the intestine by the action of **lipoprotein lipase (LPL)**, resulting in the release of **tocopherols**. Another portion is present in the form of **chylomicrons**, which are large lipoproteins rich in triglycerides. These particles regulate lipid and energy metabolism pathways. The released tocopherols are transferred to extrahepatic tissues, where they regulate additional metabolic processes.

Moreover, CLA has been shown to enhance insulin sensitivity and improve metabolism, which may help explain one of the mechanisms through which CLA reduces **adiposity**. This allows for greater uptake of fatty acids and glucose into muscle cells for energy production, accelerating fatty acid oxidation and preventing their storage as **triacylglycerols** (Houseknecht et al., 1998).

Despite the variation in proposed mechanisms regarding the effects of CLA, theories continue to differ concerning the affected biological systems, effective doses, and transport mechanisms. However, the fundamental point remains: CLA plays a significant role in reducing lipid levels and

preventing their deposition in blood vessels, which may help reduce the risk of thrombosis, atherosclerosis, or obesity (Baddini et al., 2009).

In a study using **ICR strain laboratory mice (both male and female)** fed a high-fat, CLA-enriched diet (0.5% w/w) for 30 days, fat mass was reduced by 60%. This effect was attributed to increased fat breakdown and oxidation. Similar experiments on different animal models, including rats, mice, and pigs, showed that CLA treatments at various concentrations led to decreased triglyceride levels and reduced unsaturated fatty acid content in white adipose tissue. These effects depended on the CLA dosage over a 21-day period and were primarily attributed to the **10,12-CLA isomer**, which has been shown to reduce adiposity (Park et al., 1997; Park et al., 1999).

The reduction in hepatic triglycerides and the enhanced expression of genes associated with **fatty acid oxidation**, including **peroxisome proliferator-activated receptors (PPARs)**, **acetyl-CoA oxidase**, and **Δ9-desaturase**, along with their influence on **fatty acid synthase (FAS)**, demonstrate the regulatory role of CLA in lipid metabolism. Additionally, CLA may also inhibit **HMG-CoA reductase**, a key enzyme in cholesterol biosynthesis (Purushotham et al., 2007).

Table (3-2): Effect of Commercial and Extracted Conjugated Linoleic Acid (CLA) on Cholesterol and Triglyceride Levels in Female Rats

Treatment Groups	TG ≤150 mg/dL	Ch Up to 200 mg
RAR1	127.10a	119.67a
Control	92.15c	69.54f
Commercial Grop1	118.60b	92.43c
CLA Extract Grop2	84.80d	98.91b
CLA Extract Grop3	81.82e	82.18d
CLA Extract Grop4	78.88f	78.46e

Different letters within the same column indicate significant differences at the 0.05 probability level.
The symbol **Ch** refers to total cholesterol level.
The symbol **TG** refers to triglyceride level.

3.2.2: Effect of Using Different Concentrations of Extracted Conjugated Linoleic Acid on High-Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL) Levels Compared to Commercial Linoleic Acid

The results presented in Table (3-3) demonstrate the effect of using different concentrations of extracted conjugated linoleic acid (CLA) on HDL and LDL levels. The data indicate a significant increase in HDL levels and a significant decrease in LDL levels across all administered concentrations in female laboratory animals, compared to the control group and the commercial CLA treatment.

At a 2% concentration, HDL levels increased to 54.36 mg/dL, whereas the 0.5% and 1% concentrations resulted in HDL levels of 42.70 mg/dL and 41.46 mg/dL, respectively, compared to 32.11 mg/dL in females fed a high-fat diet. In animals treated with commercial CLA, HDL levels reached 43.17 mg/dL.

Regarding LDL levels, females treated with 2% CLA showed a reduction to 44.10 mg/dL, while those treated with 1% and 0.5% concentrations had LDL values of 40.05 mg/dL and 42.99 mg/dL,

respectively, compared to 60.87 mg/dL in animals fed a high-fat diet. LDL levels in animals treated with commercial CLA were 38.42 mg/dL.

Several factors may influence LDL and HDL levels, such as high-calorie diets, obesity, and consumption of unsaturated fats. The beneficial increase in HDL is often associated with either a decrease or increase in LDL levels in the blood. The accumulation of these lipids within artery walls is prevented by HDL, which inhibits plaque formation on vascular walls, thereby preventing artery blockage and sudden thrombosis. HDL acts antagonistically to LDL in this regard.

A study by (Mohammad Reza et al., 2024) confirmed that omega supplementation increases high-density lipoprotein (HDL) and decreases low-density lipoprotein (LDL), thereby reducing the risk of thrombosis.

Additionally, enzymes play a role in regulating and dissociating triglyceride complexes, especially LDL and low-density lipoproteins derived from VLDL metabolism, which are excreted via bile, thereby reducing blood lipid levels. Failure to eliminate these lipids leads to their deposition and accumulation on arterial walls, causing vascular obstruction and increasing cardiovascular risk. Moreover, decreased activity of the desaturase 9 enzyme is associated with increased secretion of VLDL and consequently decreased triglycerides (TAG) (Sebedio, 2001).

(Franczyk-Żarowy et al., 2019) reported that feeding laboratory animals with different fat sources—including soybean oil, butter, vegetable shortening, and groups supplemented with 1% CLA—resulted in decreased TAG, HDL, and cholesterol levels after 21 days of feeding compared to groups fed soybean oil, butter, and vegetable shortening without CLA supplementation.

Table (3-3) Effect of Commercial and Purified Conjugated Linoleic Acid on LDL and HDL

Treatments	LDL ≤100 mg/Di	HDL 40-60mg/Di
RAR1	60.87a	32.11d
Control	45.76b	38.21c
Commercial %1	38.42f	43.17b
CLA %0.5	40.05e	42.70b
CLA %1	42.99d	41.46b
CLA %2	44.10c	45.36a

Different letters within the same column indicate statistically significant differences at the 0.05 probability level.

3.2.3: Effect of Using Different Concentrations of Purified Conjugated Linoleic Acid on Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) Levels Compared to Commercial CLA

The results presented in Table (3-4) indicate significant differences in ALT and AST levels. The level of ALT decreased significantly at the 2% concentration, reaching 39.5 U/L. The decrease was also notable at the 0.5% and 1% concentrations, which recorded values of 43.31 U/L and 39.43 U/L, respectively, compared to the hypercholesterolemic control group, which recorded 55.018 U/L.

Similarly, AST levels also showed a significant reduction in all treated groups, with values of 40.98, 41.91, and 45.96 U/L for the 2%, 1%, and 0.5% CLA concentrations, respectively, compared to 50.47 U/L in the high-fat diet control group.

Although CLA supplementation in a high-fat diet appears to yield beneficial effects, the long-term impact of isomer integration remains uncertain. CLA plays a role in cell signaling, activating transcription factors, and thereby regulating transcription enzymes and lipid metabolism. It also influences hepatic fat content, tocopherol levels, and serum fatty acid profiles, all of which affect adipose tissue mass, oxidative stress, and immune response (Castro-Gomez et al., 2014).

Wang et al. (2023) reported that high-fat diets, especially when supplemented with CLA, show differing effects. High-fat diets alone tend to elevate triglyceride levels and liver enzymes, potentially leading to obesity. In contrast, CLA-supplemented diets significantly enhance liver antioxidant capacity, reduce TG levels, and increase the AST/ALT ratio to 2.95%, while reducing the LA/ALA ratio to 2.58%. These changes lead to marked improvement in liver function without directly reducing obesity, indicating that a lower LA/ALA ratio is more closely associated with hepatic health than with body weight reduction.

Elevated serum levels of ALT and AST are associated with lipid metabolism and are more strongly correlated with PAI and AST than with ALT. An elevated ALT level is often an abnormal indicator of acute hepatitis, cirrhosis, autoimmune diseases, or fat accumulation in the bile duct, potentially leading to its obstruction. Furthermore, CLA impacts adiponectin metabolism, which in turn influences glucose and lipid metabolism. Intake of n-3 unsaturated fatty acids has been shown to reduce plasma lipid levels by approximately 40–50% (Ukroper et al., 2003).

Table (3-4): Effect of Commercial and Extracted Conjugated Linoleic Acid on Liver Enzymes AST and ALT

Treatments	ALT Upto48IU/L	AST UPTO48IU/L
RAR1	54.47a	55.18a
Control	46.12b	40.59c
Commercial %1	38.46d	40.80c
CLA %0.5	45.96b	43.31b
CLA %1	41.91c	39.43d
CLA %2	40.98c	39.15d

Different letters within the same column indicate significant differences at the 0.05 probability level.

REFERENCES

Zongo, K., Krishnamoorthy, S., Moses, J. A., Yazici, F., Çon, A. H., & Anandharamakrishnan, C. (2021). Total conjugated linoleic acid content of ruminant milk: The world status insights. *Food Chemistry, 334*, Article 127605. https://doi.org/10.1016/j.foodchem.2020.127605.

Duchemin, S., Bovenhuis, H., Stoop, W. M., Bouwman, A. C., van Arendonk, J. A. M., & Visker, M. H. P. W. (2013). Genetic correlation between composition of bovine milk fat in winter and summer, and DGAT1 and SCD1 by season interactions. *Journal of Dairy Science, 96*(1), 592–602. https://doi.org/10.3168/jds.2012-5640

Basak, S., & Duttaroy, A. K. (2020). Conjugated linoleic acid and its beneficial effects in obesity, cardiovascular disease, and cancer. Nutrients, 12(11), 3299. https://doi.org/10.3390/nu12113299

Vaisar, T., Wang, S., Omer, M., Irvin, A. D., Storey, C., Tang, C., & den Hartigh, L. J. (2022). 10,12-conjugated linoleic acid supplementation improves HDL composition and function in mice. *Journal of Lipid Research, 63*, 100235. https://doi.org/10.1016/j.jlr.2021.100235

Han, S. N., Leka, L. S., Lichtenstein, A. H., Ausman, L. M., Schaefer, E. J., & Meydani, S. N. (2002). Effect of hydrogenated and saturated, relative to polyunsaturated, fat on immune and inflammatory responses of adults with moderate hypercholesterolemia. *Journal of Lipid Research, 43*(3), 445–452.

Khodadadi, E., Khodadadi, E., Chaturvedi, P., & Moradi, M. (2020). Cholesterol review: A metabolically important molecule. Endocrine Practice, 26(12), 1514–1523. https://doi.org/10.4158/EP-2020-0347

Levin, B. E., & Dunn-Meynell, A. A. (2002). Defense of body weight depends on dietary composition and palatability in rats with diet-induced obesity. Am J Physiol Regul Integr Comp Physiol, 282(1), R46-54. https://doi.org/10.1152/ajpregu.2002.282.1.R46

Abdul Kadir, N. A. A., Rahmat, A., & Jaafar, H. Z. (2015). Protective effects of tamarillo (Cyphomandra betacea) extract against high fat diet i **Van Pelt, L. F. (1977)**. Ketamine and xylazine for surgical anesthesia in rats. J Am Vet Med Assoc, 171(9), 842-844 nduced obesity in Sprague-Dawley rats. Journal of obesity, 2015.

Siedel , J. ; Schlumberger , H. ; Klose , S. ; Ziehenhorn , J. and Wahleteld , A.W. (1981) . Improved reagent for the enzymatic determination of serm cholesterol. J. Clin. Chem. Clin. Biochem. , 19 (8) : 838 – 839

Fossati, P. and Prencipe, L. (1982). Serum triglyceridese determined colorimetrically with an enzyme that produces hydrogyen peroxide. Clin Chem.,28: 2077.

Finley, P.R; Schifman, R.B; Williams, R.J. and Lichti, D.A. (1978). Cholesterol in high density lipoprotein : use of mg2+ / dextron sulfate in its enzymatic measurement . Clin. Chem.,24 : 931-933 .

Reitman and Frankal . (1957) . A colorimetric method for the determination of serum glutamic oxalaloacetic and glutamic pyruvic transaminases , Am . J. clin. Patho.,28 : 56-59

Park, Y., Albright, K. J., Liu, W., Storkson, J. M., Cook, M. E., & Pariza, M. W. (1997). Effect of conjugated linoleic acid on body composition in mice. Lipids, 32(8), 853–858.
Houseknecht, K.L., Vanden-Heuvel, J.P., Moya- Camarena S.Y., Portocarrero, C.P., Peck, L.W., Nickel, K.P. & Belury, M.A. (1998) dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker diabetic fatty fa/fa rat. Biochemical and Biophysical Research Communications. 244: 678-682. **Baddini Feitoza, A., Fernandes Pereira, A., Ferreira da Costa, N., & Gonçalves Ribeiro, B. (2009).** Conjugated linoleic acid (CLA): Effect modulation of body composition and lipid profile. *Nutrición Hospitalaria, 24*(4), 422–428.

Park, Y., Storkson, J. M., Albright, K. J., Liu, W., & Pariza, M. W. (1999). Evidence that the trans-10,cis-12 isomer of conjugated linoleic acid induces body composition changes in mice. Lipids, 34(3), 235–24 **Purushotham, A., Shrode, G. E., Wendel, A. A., Liu, L. F., & Belury, M. A. (2007).** Conjugated linoleic acid does not reduce body fat but decreases hepatic steatosis in adult Wistar rats. *Journal of Nutritional Biochemistry, 18*(10), 676–684.

Sebedio, J. L., Angioni, E., Chardigny, J. M., Gregoire, S., Juaneda, P., & Berdeaux, O. (2001). The effect of conjugated linoleic acid isomers on fatty acid profiles of liver and adipose tissues and their conversion to isomers of 16:2 and 18:3 conjugated fatty acids in rats. *Lipids, 36*(6), 575–582.

Franczyk-Żarowy, M., Kuś, E., & Kostogrys, R. B. (2019). Effect of conjugated linoleic acid and different type of dietary fat on serum lipid profile, liver enzymes activity and oxidative stress markers in Wistar rats. Rocz Panstw Zakl Hig, 70(1), 27-33. doi: 10.32394/rzph.2019.0049.
Castro-Gomez P, Fontecha J, Rodriguez-Alcala LM. 2014. A highperformance direct transmethylation method for total fatty acids assessment in biological and foodstuff samples. Talanta 128:518 –523.
https://doi.org/10.1016/j.talanta.2014.05.051.

Wang, K., Xin, Z., Chen, Z., Li, H., Wang, D., & Yuan, Y. (2023). Progress of Conjugated Linoleic Acid on Milk Fat Metabolism in Ruminants and Humans. Animals, 13(21), 3420. https://doi.org/10.3390/ani13213429.

Ukropec, J., Reseland, J. E., Gasperikova, D., Demcakova, E., Madsen, L., Berge, R. K., Rustan, A. C., Klimes, I., Drevon, C. A., & Sebökova, E. (2003). The hypotriglyceridemic effect of dietary n-3 FA is associated with increased ß-oxidation and reduced leptin expression. *Lipids, 38*(10), 1023–1029

Martha, R., Toharmat, T., Rofiah, N., & Anggraeni, D. (2019). Comparison of extraction methods for fatty acid and conjugated linoleic acid quantification in milk. In *IOP Conference Series: Materials Science and Engineering* (Vol. 546, p. 042022). IOP Publishing. https://doi.org/10.1088/1757-899X/546/4/042022

Mohsen, A. H., & Mohsen, I. H. (2019). Effect of black seed oil on some physiological parameters in female rats treated with aflatoxin B1, B2. *Indian Journal of Public Health Research and Development, 10*(10), 2822–2827.

Mohsen, I. H., & Mohsen, A. H. (2019). Study of the influence of the biochemical parameters of laboratory rats treated with aflatoxin and black seed oil. *Indian Journal of Forensic Medicine and Toxicology, 13*(4), 1003–1008.

Al-Sallami, N. Y., Harjan, A., & Harjan, I. (2017). The effect of antibacterial agent produced from *Penicillium griseofulvum* in inhibiting growth of bacteria and in some physiological and biochemical parameters of blood in male white rats. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences, 19*(4), 892–896.