The potential hypercholesterolemic effect of feeding rats on improved nontraditional white soft cheese supplemented with vegetable oils and starter cultures

By

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Abstract

The present work was done on the health promoting power for lowering the blood cholesterol by feeding the rate on advanced non-traditional soft white cheese with vegetable oils and starter cultures. The experimental included 36 female albino rates fed on basal diet for one week and then divided into 6 groups two of them were served as positive and negative controls, The other 4 groups were fed for 6 weeks on the above mentioned cheese treatments. The non-traditional white soft cheeses were more effective in lowering serum total cholesterol especially T3 (3% starter culture). The serum triglycerides level, serum VLDL + LDL level, The TC/HDL and LDL/HDL ratios were showed a pronounced decrease as the time of feeding progressed. All the blood serum HDL cholesterol were also gradually decreased. Serum glutamic oxalacetic transaminase (GOT) level showed a significantly low activity whereas, T3 was the lowest. On the other hand the serum glutamic pyrovatic transaminase (GPT) level were observed to have a significant decrease in the serum levels of this enzymes relative to the negative control treated group. Hence the non-traditional white soft cheese made with vegetable oils and starter culture copuld be better protect against coronary heart disease.

Keywords: soft white cheese, non-traditional cheese, starter culture, CBS, GOT, GPT

Introduction

Thirty to fifty percent of the dietary intake is normally derived from fat. Fat is largely composed of triglycerides; only a small proportion is present as free fatty acids, cholesterol esters and phospholipids. Vegetable oils are rich in poly unsaturated fatty acids. Excessive and repeated heating of these oils and whole mixed milk, or hydrogenation causes changes in these oils and whole mixed milk. Because of fats are insoluble in water they are transported in the plasma with proteins as a complex. There are four main lipoprotein fractions: chylomicron, pre- β -lipoprotein

(very low density lipoprotein VLDL), β -lipoprotein (low density lipoprotein LDL) and α -lipoprotein (high density lipoprotein HDL). Each has a specific role. In contrast most of the cholesterol (60–70%) is carried by LDL which has higher protein content and so is heavier and denser.

Lowering the serum cholesterol in hypercholesterolemia can delay the development of the atherosclerosis and reduce the risk of heart disease. (Anonymous, 1984).

Cheese consumption has been steadily increasing over the past 20 years (Putnam & Allshouse 1999). Also, cheese considers being the main sources of animal fat and dietary cholesterol, since over 60% of dairy fat is saturated. The dietary cholesterol is essential for membrane structure, hormones and steroid biosynthesis (Adanyi & Varadi, 2003; Rozner & Garti, 2006). It has been recognized that its elevated levels in plasma are directly correlated to increase cardiovascular heart diseases (Okazaki et al., 2006). Dietary milk fats, on account of their higher content of saturated fatty acids, have long been associated with a variety of human diseases; however, recent studies have focused on the healthy components of milk fats, a decline in consumption of dairy-derived products (rich in saturated fat increases serum total and LDL-cholesterol concentrations) has occurred during the last decade because of their negative health image, as well as, an increase of vegetable fats which consider to be rich in phytosterols and unsaturated fatty acids in the daily food intake. This may give new opportunities to control elevated serum cholesterol concentration, which is known to be one of the most important risk factor for atherosclerotic vascular diseases (Assmann et al., 1999).

So, this work was planned to explain the effect of the improved non-traditional white soft cheese using vegetable oils and different ratios of starter culture on rats fed on cholesterol-enriched diet via biochemical examinations *i.e.* (total cholesterol, triglycerides, HDL-cholesterol, VLDL, LDL, GOT and GPT)

Materials and Methods

1. Materials:

1.1. Raw milk:

Fresh mixed milk (cow and buffalo's, 1:1) used in this study was obtained from the herd of Faculty of Agriculture, Moshtohor, Benha University, Egypt.

1.2. Skim milk powder (SMP):

Low heat skim milk powder was purchased from local market, which imported from California Dairies, Inc, Fresno, California, USA.

1.3. Cocoa butter substitute:

Super "ERCOAT CBS" cocoa butter substitute was obtained from International Egyptian Food Company (IEFCO Egypt), Attaqa, Suez, Egypt.

1.4. Starter culture:

Pure strain of *Lactobacillus casei* NCAIM B01137 was obtained from National Collection Agricultural Institute of Microbiology. Starter culture contains *Lactococcus lactis* ssp. *lactis* and *Lb. delbrueckii* ssp. *bulgaricus* was obtained from Chr. Hansen's Laboratories, Horsholm, Denmark.

1.5. Stabilizer:

Lacta-815 was obtained from Misr Food Additives (MIFAD) Company, Giza, Egypt.

1.6. Salt:

Commercial pure fine grade salt (NaCl) was obtained from the Egyptian Salt & Minerals Company (EMISAL), Egypt.

1.7. Rennet:

Microbial rennet powder (Formase TL2200) was obtained from Chr. Hansen's Laboratories, Copenhagen, Denmark.

1.8. Calcium chloride:

Calcium chloride (CaCl₂) was obtained from El-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt.

1.9. The diagnostic kits:

There were obtained from Sentinel CH. Millan, Italy and purchased from Technogene Company.

1.10. Experimental rats:

Thirty sex female albino rats (90–100 g) were obtained from animal house of Crops Tech. Department, Food Technology Research Institute, Agriculture Research Center. All rats were fed on basal diet Table 2 (20g per day/ rat) for one week (adaptation period) and then divided randomly into 6 groups (6 rats/each) to carry the experiment of biological evaluation. *The first* group was fed on basal diet (cholesterol free diet) throughout the experimental period (7 weeks) and was considered to be as negative control. *The second* group was fed on basal diet contained 0.5% cholesterol (cholesterol–enriched diet) and considered as positive control. *The other four* groups were fed on a basal diet contained 0.5% cholesterol (cholesterol–enriched diet) to create hypercholesterolemic rate for one week, then they were fed for six weeks on a cholesterol–enriched diet supplemented with different cheese treatments according to the chemical composition from T1 to T4 as described in Table (1).

The weight of each rat was recorded weekly and blood samples were collected by withdrawing each two weeks from vein plexus eye, centrifuged at 3000 rpm to obtain the blood serum, which stored at (-20°C) for biochemical assay. On the other hand, by the end of the experiment (7 weeks), rats were sacrificed and the blood was collected in clean test tubes and centrifuged to obtain the serum.

Table (1): The experimental rat groups and their diets.

	Group	Experimental diets (per 6 rats)				
Cholesterol free diet	Negative control	120g basal diet [•]				
iet	Positive control	119.4g basal diet + 0.6g cholesterol				
Cholesterol–enriched diet	Treatment 1	96.8g basal diet* + 0.6g cholesterol + 22.6g cheese (control cheese)				
sterol-e	Treatment 2	101.7 basal diet* + 0.6g cholesterol + 17.8g (cheese T1)				
Chole	Treatment 3	101.7 basal diet* + 0.6g cholesterol + 17.8g (cheese T2)				
	Treatment 4	101.7 basal diet* + 0.6g cholesterol + 17.8g (cheese T3)				

basal diet*: as mentioned in Table (2) basal diet*: free from casein and fat

Table (2): The composition of the basal diet, the minerals and vitamins formula added to the basal diet.

Ingredient	%	Minerals mixture		Vitamins mixture		
		Minerals	Conc.	Vitamins	Conc.	
Casein	10.00	CaCO ₃	600g	Vit A	2000 IU	
Tallow	9.25	K ₂ HPO4	645g	Vit D	200 IU	
Corn oil	0.75	CaHPO ₄ .2H ₂ O	150g	Vit E	10 IU	
Cellulose	10.00	MgSO ₄ .2H ₂ O	204g	Vit K	10 mg	
Bile acid	0.25	NaCl	334g	Thiamin	0.5 mg	
Mineral mix	4.00	$Fe(C_6H_5O_7).6H_2O$	55g	Pyridoxine	0.5 mg	
Vitamin mix	1.00	KI	1.6g	Panthothenic acid	4.0 mg	
Starch	64.75	MnSO ₄ .4H ₂ O	10g	Riboflavin	0.8 mg	
		ZnCl ₂	0.5g	Niacin	4.0 mg	
		CuSO ₄ .5H ₂ O	0.6g	Choline chloride	200 mg	
				Inositol	10 g	
				P amino benzoic acid	10 mg	
				VitB ₁₂	0.03 mg	

		Biotin	0.02 mg
		Folic acid	0.02 mg

• AOAC (1998).

2. Methods:

2.1. Cheese manufacture:

Non-traditional white soft cheese was manufactured by using the method as described in Fig (2); however, the control cheese (traditional Domiati cheese) was made according to **Fahmi and Sharara (1950).**

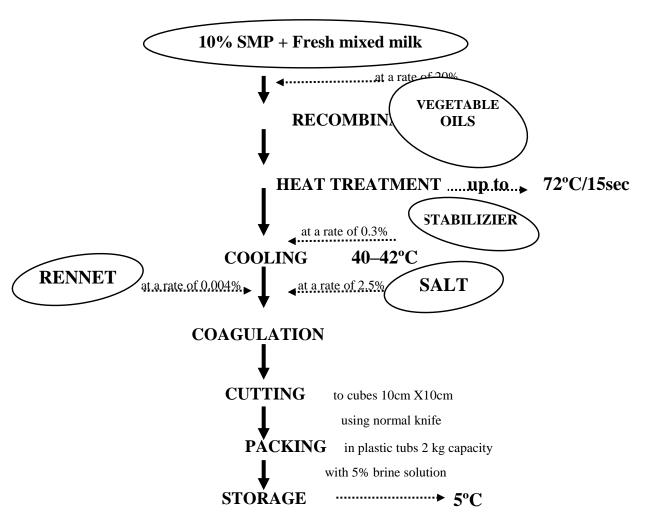


Fig (1): Flow diagram of making white soft cheese using non-traditional method from skim milk powder and vegetable oils

2.2. Biological analysis:

2.3. Determination of blood serum total cholesterol:

Total cholesterol was determined according to the method of **Richmond** (1973).

2.4. Determination of blood serum triglycerides:

Triglycerides were determined according to the method of Fassati and Principe (1982).

2.5.. Determination of serum high-density lipoprotein cholesterol (HDL-cholesterol):

HDL-cholesterol was determined according to the method of Gordon (1977).

2.6. Determination of serum low-density lipoprotein cholesterol (LDL-cholesterol):

LDL-cholesterol was calculated using the method of **Hatch and Lees (1968).**

2.7. Determination of serum transaminases activity:

Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were determined according to the method described by **Reitman and Frankel** (1957).

Results and Discussion

Blood serum total cholesterol level:

Table (3) and Fig (2) show the changes of serum total cholesterol level (mg/dl) of rats as a result of feeding on cholesterol—enriched diets containing improved non—traditional white soft cheeses with different ratios of starter culture. At the commencement there were differences appeared in the total cholesterol level between the positive control group and all other groups.

The serum total cholesterol concentration of positive control treated group was 98.14 mg/dl in the first week and increased to be 146.43 mg/dl at the end of feeding period (6 weeks).

In contrast, the total cholesterol concentration increased in the rat groups fed on cholesterol-enriched diet containing supplementation of cheese treatment, whereas, it was 101.14, 99.71 and 97.29 mg/dl and increased to be 115.43, 116.00 and 108.00 mg/dl for T1 to T3 after 2 weeks of feeding, respectively. By increasing the feeding period these values start to decrease after 4 weeks to be 104.86, 101.43 and 86.86 mg/dl in the same order. The decrease of total cholesterol was continued to be 97.14, 92.00 and 81.14 mg/dl at the end of feeding period for T1, T2 and T3, respectively. On the other hand, the serum total cholesterol in group C was increased periodically then decreased by the end of feeding period and recorded 102.86, 131.29, 124.71 and 109.57 mg/dl at 0, 2, 4 and 6 weeks of feeding, respectively. Also, from the obtained results it could be noticed that improved non-traditional white soft cheeses supplemented with vegetable oils and starter cultures were more effective for lowering blood serum total cholesterol levels. The lowest level of total cholesterol of all the treatments and recorded 81.14 mg/dl fot T3 by the end of feeding period. These results are in accordance with the results reported by El-Alfy *et al.*, (2004).

From the statistical point of view the results of blood serum total cholesterol levels indicated significant differences either for feeding periods or as a result of applying different treated groups.

Blood serum triglycerides level:

Table (3) and Fig (2) show the changes of serum triglycerides level (mg/dl) of rats fed on cholesterol—enriched diet containing improved non–traditional white soft cheeses with different ratios of starter culture. At the beginning of the feeding period there was no pronounced difference between negative control group, positive control group and the supplementary groups in blood serum triglycerides. These values were 69.35, 71.98, 73.03, 71.28, 71.11 and 72.68 mg/dl for negative control, positive control, C, T1, T2 and T3, respectively. The results are in agreement with **During** *et al.*, (2000) who stated that the unsaturated fat enriched cheese induced a slight decrease (16%) of serum triglycerides concentrations compared with the traditional cheese.

Serum triglycerides level of both negative control group and positive control group increased gradually by increasing the feeding period to be 77.41and 91.30 mg/dl, respectively by the end of the experiment (6 weeks). On the other hand, the serum triglycerides level decreased for all treatments during the interval feeding period and recorded 70.41, 66.90, 62.97 and 60.42 mg/dl for supplementary groups C, T1, T2 and T3 in order, by the end of the feeding period. Also, from the obtained results it could be noticed that the lowest triglycerides value was recorded for T3 followed by T2; this is due to the effect of the different ratios of starter culture, (3% and 2%). The obtained results are in agreement with **El-Alfy** *et al.*, (2004) and Gafour (2005) who reported that rats feeding on probiotic UF Feta-like cheese decreased the triglycerides level after 2 weeks of feeding.

Statistical analysis of blood serum triglycerides level indicated that there were significant differences among treated groups.

Blood serum HDL-cholesterol level:

The changes of serum HDL-cholesterol (mg/dl) of rats feeding on cholesterol-enriched diets containing improved non-traditional white soft cheese with different ratios of starter culture are show in Table (3) and Fig (4). The blood serum HDL-cholesterol recorded 38.05, 38.29, 39.17, 37.63, 37.44 and 38.8 mg/dl for negative control, positive control, C, T1, T2 and T3, respectively. All the blood serum HDL-cholesterol values decreased gradually in all the experimental groups by progressing of feeding time.

By the end of the feeding period (6 weeks) the serum HDL-cholesterol level was 36.42, 36.14, 36.02, 33.85, 34.46 and 36.58 mg/dl for negative group, positive group, C, T1, T2 and T3, respectively. From such results it could be concluded that HDL-cholesterol level of treatment 3 was slightly higher than other treated groups. This reflects the effect of either vegetable oils or starter cultures of improved non-traditional white soft cheese (T3) as it contains 3% of starter. The obtained results indicated also that the high decrease of blood serum HDL-cholesterol was in all groups fed on diet contains cheeses. Similar results also were obtained by **Ibrahim (2002)**.

The statistical analysis showed that there were non significant differences for blood serum HDL-cholesterol level among all treated groups.

Table (3): The hypercholesterolemic effect of rats fed on cholesterol-enriched diet containing improved non-traditional white soft cheeses.

Feeding	g		Treated ;	groups				
period (week)	- 10 5 000-10	Positive Control	С	T 1	T2	Т3		
		Blood serum total cholesterol (mg/dl)						
0 92.86 ^{EFG}		98.14 ^{EFG}	102.86 ^{D-G}	101.14 ^{D-G}	99.71 ^{F-G}	97.29 ^{EFG}		
2	93.86 ^{EFG}	135.43 ^{ABC}	131.29 ^{BCD}	115.43 ^{C-F}	116.00 ^{C-F}	$108.00^{\text{C-G}}$		
4	93.57 ^{EFG}	142.86^{AB}	$124.71^{\text{B-E}}$	104.86^{D-G}	101.43 ^{D-G}	86.86 ^{FG}		
6	96.71 ^{EFG}	146.43 ^A	109.57 ^{C-G}	97.14 ^{F-G}	92.00^{EFG}	81.14^{G}		
Mean	94.25	130.72	117.11	104.64	102.29	93.32		
L.S.D at 5%:	Treated groups = 13.092	Feeding po	eriod = 10.621	Treated grou	ps × Feeding perio	od = 26.017		
		Blood	serum trigly	ycerides (mg	g/dl)			
0	69.35 ^B	71.98 ^B	73.03 ^B	71.28 ^B	71.11 ^B	72.68 ^A		
2	74.96 ^A	83.01 ^A	71.10^{B}	70.05^{B}	70.75^{B}	69.35 ^B		
4	75.48 ^A	88.62 ^A	70.64^{B}	68.31 ^D	70.05^{B}	67.95 ^D		
6	77.41 ^A	91.30 ^A	70.41^{B}	66.90^{E}	62.97^{D}	60.42^{E}		
Mean	74.30	83.73	71.30	69.14	68.72	67.60		

 $\begin{array}{lll} L.S.D \ at \ 5\%: & Treated \ groups = 0.016 \\ Feeding \ period & = N.S \ (Non \ Significant) \\ Treated \ groups & \times \ Feeding \ period & = N.S \ (Non \ Significant) \\ \end{array}$

		Blood se	erum HDL-	-cholesterol	l (mg/dl)				
0	38.05 ^A	38.29 ^A	39.17 ^A	37.63 ^A	37.44 ^A	38.80^{A}			
2	37.66 ^A	37.63 ^A	38.47^{A}	36.60^{A}	36.04 ^A	38.38^{A}			
4	37.19 ^A	36.98^{A}	36.23^{A}	35.67 ^A	35.86 ^A	37.16^{A}			
6	36.42 ^A	36.14 ^A	36.02 ^A	33.85 ^A	34.46 ^A	36.58 ^A			
Mean	37.33	37.26	37.47	35.94	35.95	37.73			

L.S.D at 5%: Treated groups = N.S (Non Significant) Feeding period = N.S (Non Significant) Treated groups × Feeding period = N.S (Non Significant)

Blood serum VLDL + LDL-cholesterol (mg/dl)								
0	54.81	59.85	63.69	63.51	62.27	58.49		
2	56.20	97.80	92.82	78.83	79.96	69.62		
4	56.38	105.88	88.48	69.19	65.57	49.70		
6	60.29	110.29	73.55	63.29	57.54	44.56		
Mean	56.92	93.46	79.64	68.71	66.34	55.59		
		То	tal choleste	rol/HDL rati	io			
0	2.44	2.56	2.63	2.69	2.66	2.51		
2	2.49	3.60	3.41	3.15	3.22	2.81		
4	2.52	3.86	3.44	2.94	2.83	2.34		
6	2.66	4.05	3.04	2.87	2.67	2.22		
Mean	2.53	3.52	3.13	2.91	2.85	2.47		

Table (3): Continued.

Feeding period (week)			Treated	groups				
	Negative Control	Positive Control	С	T1	T2	Т3		
	LDL/HDL ratio							
0	1.44	1.56	1.63	1.69	1.66	1.51		
2	1.49	2.60	2.41	2.15	2.22	1.81		
4	1.52	2.86	2.44	1.94	1.83	1.34		
6	1.66	3.05	2.04	1.87	1.67	1.22		
Mean	1.53	2.52	2.13	1.91	1.85	1.47		

(-ve): basal diet (cholesterol free diet)

(+ve): cholesterol-enriched diet

C: cholesterol-enriched diet supplemented with control cheese without vegetable oil or starter culture

T1: cholesterol—enriched diet supplemented with cheese with vegetable oil and 1% starter culture
T2: cholesterol—enriched diet supplemented with cheese with vegetable oil and 2% starter culture
T3: cholesterol—enriched diet supplemented with cheese with vegetable oil and 3% starter culture

Values with the same letters are non significant different.

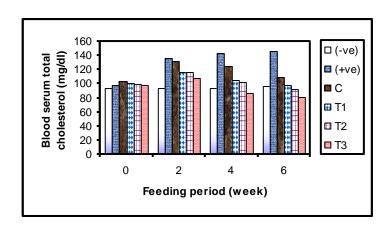


Fig. (2): Blood serum total cholesterol level (mg/dl) of rats fed on cholesterol-enriched diet containing improved non-traditional white soft cheeses

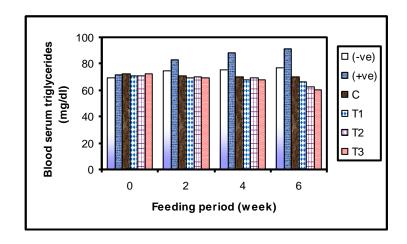


Fig (3): Blood serum triglycerides level (mg/dl) of rats fed on cholesterol-enriched diet containing improved non-traditional white soft cheeses

Blood serum VLDL+LDL-cholesterol level:

Table (2) and Fig (4) illustrate the changes of serum VLDL + LDL cholesterol (mg/dl) of rats as affected by cholesterol—enriched diets containing improved non—traditional white soft cheese with different ratios of starter culture, respectively. These values started to increase during the interval feeding period; this increase was more noticeable in positive group. However, the serum VLDL + LDL cholesterol values of groups fed on cheeses using starter culture cleared pronounced decrease especially that group fed on cheese using 3% of starter culture which recorded a decrease from 58.49 mg/dl in zero time to 44.56 mg/dl at the end of feeding period, followed by T2, T1 then C group. This gives an indicator about starter culture effect on lowering this parameter. These results are in agreement with **During et al., (2000a),** who mentioned that, the partial substitution of milk fat by vegetable oils in soft-ripened cheese resulted a decrease of blood serum LDL—cholesterol.

Total cholesterol/HDL and LDL/HDL ratios:

TC/HDL and LDL/HDL ratios are given in Table (3) and illustrate by Fig (6), respectively. A significant increase in total cholesterol/HDL and LDL/HDL ratios was observed in positive control fed group and treatment C, which has an effect on cardiovascular diseases.

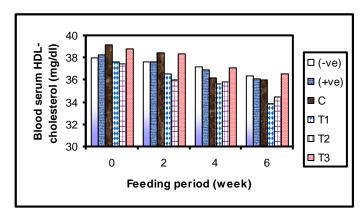


Fig. (4): Blood serum HDL-cholesterol level (mg/dl) of rats fed on cholesterolenriched diet containing improved non-traditional white soft cheeses

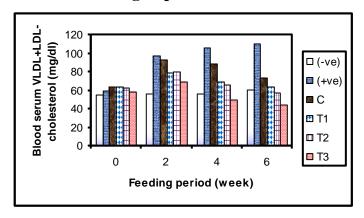


Fig. (5): Blood serum VLDL+LDL-cholesterol level (mg/dl) of rats fed on cholesterolenriched diet containing improved non-traditional white soft cheeses

The cholesterol–enriched diet supplemented with improved non–traditional white. It could be noticed that the negative control was lower in VLDL + LDL than the positive control and the other supplemented dietary groups at the beginning of feeding period and recorded 54.81, 59.85, 63.69, 63.51, 62.27 and 58.49 mg/dl for negative group, positive group, C, T1, T2 and T3, soft cheese with different ratios of starter culture groups resulted in the decrease of a number of proatherogenic factors, such as TC/HDL and LDL/HDL (Morise *et al.*, 2004).

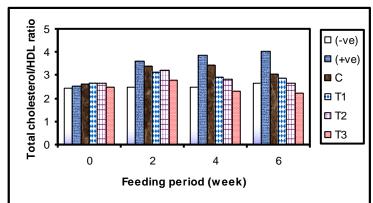


Fig. (6): Total cholesterol/HDL ratio of rats fed on cholesterol—enriched diet containing improved non-traditional white soft cheeses

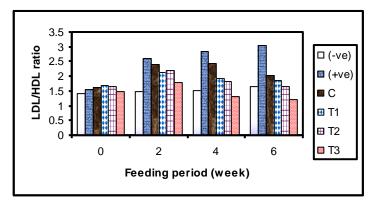


Fig. (7): LDL/HDL ratio of rats fed on cholesterol—enriched diet containing improved non-traditional white soft cheeses

During *et al.*, (2000) reported a significant decrease of the LDL/HDL ratio of rats fed experimental cheeses, considered as an index of CVD risk. Moreover, TC/HDL and LDL/HDL ratios are also predictors of coronary risk (National Cholesterol Education Program Expert Panel, 1994).

Liver functions:

Liver functions can be measured through the liver enzymes; these enzymes are groups of clinical biochemistry laboratory blood assays to give information about the state of liver. Hepatic liver involvement in some diseases can be of crucial importance. The liver enzymes tests preformed by a medical technologist on a serum or plasma samples. Some of tests are associated with functionality, (e.g. albumin); some with cellular integrity, (e.g. transaminase) and some with conditions linked to the biliary tract (gamma—glutamyl transferase and alkaline phosphatase). Several biochemical tests are useful in the evaluation and management of hepatic dysfunction. These tests can be used to:-

- A- Detect the presence of liver disease.
- B- Distinguish among different types of liver disorders.
- C- Gauge the extent of known liver damage.
- D- Follow the response to treatment.

Glutamic oxaloacetic transaminase (GOT) is a pyridoxal phosphate dependent enzyme which exists in cytoplasmic and mitochondrial forms. GOT plays a role in amino acids metabolism, urea and tricarboxylic acid cycles, while glutamic pyruvic transaminase (GPT) catalyzes the two parts of the alanine cycle, it catalyzes the transfer of an amino group from alanine to $\acute{\alpha}$ -ketoglutarate. The products of this reversible transamination reaction being pyruvate and glutamate. So, this part was to measure the GOT and GPT of hypercholesteremic rats fed on improved non-traditional white soft cheeses.

Serum glutamic oxaloacetic transaminase (GOT) level:

Table (4) and Fig (8) show the changes in serum glutamic oxaloacetic transaminase (GOT) levels (U/ml) of rats as a results of feeding on cholesterol–enriched diets containing improved non–traditional white soft cheese with different ratios of starter culture. From such results it could be observed that serum GOT level for all rat groups increased allover the feeding period to be 61.11, 74.05, 65.83, 66.03, 70.00 and 54.76 U/ml for negative group, positive group, C, T1, T2 and T3, respectively at the 4th week of feeding period.

By the end of the feeding period (6 weeks), the serum glutamic oxaloacetic transaminase (GOT) level recorded 60.24, 82.22, 73.05, 63.81, 50.95 and 45.08 U/ml for negative group, positive group, C, T1, T2 and T3, respectively. These results showed an increase of GOT activities in positive group, negative group and control (C) treatments, while the supplemented dietary groups indicated a decrease in activity of this enzyme .T3 showed low activity of this enzyme compared to other groups, followed by T2 and T1 compared with the control.

This observation indicate that fatty infiltration and degeneration of liver cells caused by cholesterol feeding were significantly reduced by cholesterol-enriched diet supplemented with improved cheese with vegetable oils and starter cultures, similar results were recorded by **Sandhya** and **Rajamohan** (2008).

Significant differences were recorded for serum glutamic oxaloacetic transaminase (GOT) level as a result of different treatments. Different feeding periods caused significant difference in GOT level and this was clear from the analysis of variance at level of 5% with LSD 0.017 and 0.014 for the treated groups and feeding period, respectively.

Serum glutamic pyruvic transaminase (GPT) level:

The results obtained for serum glutamic pyruvic transaminase (GPT) level of rats feeding on cholesterol–enriched diets containing improved non–traditional white soft cheese with different ratios of starter culture (Table, 4 and Fig 9) show that serum GPT enzyme level was almost the same for all rat groups at the beginning of feeding period.

The serum glutamic pyruvic transaminase level of different groups was slightly increased during feeding period as it was 29.47, 44.75, 41.61, 30.79, 36.92 and 34.38 U/ml for negative group, positive group, C, T1, T2 and T3, respectively, at the end of feeding period.

From these results, it could be noticed that the negative group had low activity of this enzyme compared with other groups. The cholesterol–enriched diet supplemented with vegetable oils and starter cultures fed groups were also observed to have a significant decrease in the serum levels of this enzyme compared with the negative control treated group.

Oluba *et al.*, (2008) stated that the palm oil–fed rats group; was also observed to have a significant decrease in the serum levels of these enzymes.

Analysis of variance at level of 5% for GPT indicated that there were highly significant differences between either positive, negative groups and all the treated groups either at zero time or during the intervals feeding period with LSD 0.012 and 0.009 for the treated groups and feeding period, respectively.

Table (4): Liver functions of rats fed on cholesterol—enriched diet containing improved non-traditional white soft cheeses.

	Feeding Treated groups							
	period (week)	Negative Control	Positive Control	С	T1	T2	Т3	
		Serum	gluatamic ox	aloacetic tra	nsaminase (GOT) level (U/ml)	
	0	50.71 ^E	51.67 ^E	51.67 ^E	51.43 ^E	51.19 ^E	51.67 ^E	
	2	61.90^{D}	72.86 ^A	64.05^{B}	68.25^{B}	61.90^{D}	60.95^{D}	
	4	61.11 ^D	74.05 ^A	65.83 ^B	66.03 ^B	70.00^{B}	54.76^{E}	
	6	60.24^{D}	82.22^{A}	73.05^{B}	63.81 ^B	50.95^{E}	45.08^{E}	
	Mean	58.49	70.20	63.65	62.38	58.51	53.12	
L.S.D at 5%:	Treated grou	ps = 0.017	Feeding period = 0	.014 Treated group	s × Feeding period	= N.S (Non Signific	eant)	
		Seru	m gluatamic p	yruvic tran	saminase (G	PT) level (U	/ml)	
	0	27.11 ^R	27.96 ^{OP}	28.81 ^M	28.15 ^o	28.15°	27.68 ^{PQ}	
	2	27.77^{FQ}	28.53 ^N	28.91^{M}	31.55 ^I	31.17^{J}	33.62^{G}	
	4	28.95 ^Q	39.85^{D}	40.16 ^C	33.06^{H}	41.36^{B}	40.42^{C}	
	6	29.47 ^L	44.75 ^A	41.61 ^B	30.79^{K}	36.92^{E}	34.38^{F}	

Feeding period = 0.009Treated groups \times Feeding period = 0.024

(-ve): basal diet (cholesterol free diet) (+ve): cholesterol-enriched diet

C: cholesterol-enriched diet supplemented with control cheese without vegetable oil or starter culture

T1: cholesterol-enriched diet supplemented with cheese with vegetable oil and 1% starter culture

T2: cholesterol—enriched diet supplemented with cheese with vegetable oil and 2% starter culture

T3: cholesterol-enriched diet supplemented with cheese with vegetable oil and 3% starter culture

Values with the same letters are non significant different.

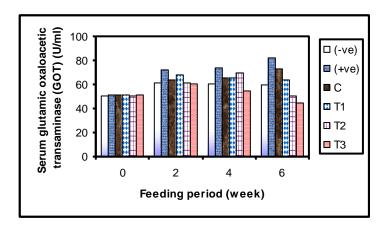


Fig. (8): Serum glutamic oxaloacetic transferase (GOT) level (U/ml) of rats fed on cholesterol—enriched diet containing improved non—traditional white soft cheeses

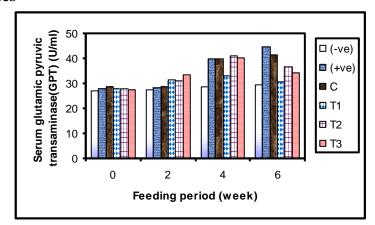


Fig. (9): Serum glutamic pyruvic transferase (GPT) level (U/ml) of rats fed on cholesterol—enriched diet containing improved non—traditional white soft cheeses

In conclusion, the data generated in this work showed clearly that the non-traditional white soft cheese made from vegetable oils and starter cultures consumption could be better protection against coronary heart disease risk than cheese without vegetable oils supplemented with starter cultures and therefore not all dietary fats generally classified as saturated raise serum cholesterol concentration thus such foods have a place in our daily diets.

REFERENCES

Adanyi, N. and Varadi, M. (2003). Development of organic phase amperometric biosensor for measuring cholesterol in food samples. Euro. Food Res. & Tech., 218(1):99–104.

- **Anonymous.** (1984). Lipid Research Clinic's Coronary Primary Prevention. Trial results I and II. J. Amer. Med. Ass., 251(3):351–374.
- **AOAC.** (1998). Official Methods of Analysis, 15th ed., Association of Official Analytical Chemists, Inc., Arlington, Virginia, USA.
- **Assmann, G.; Cullen, P.; Jossa, F.; Lewis, B. and Mancini, M. (1999).** Coronary heart disease: reducing the risk: the scientific background to primary and secondary prevention of coronary heart disease. A worldwide view. International task force for the prevention of Coronary Heart Disease. Arterioscler. Thromb. Vasc. Biol., 19:1819–1824.
- **During, A.; Combe, N.; Mazette, S. and Entressangles, B. (2000).** Effects on cholesterol balance and LDL cholesterol in the rat of soft-ripened cheese containing vegetable oils. J. Am. Coll. Nutr., 19(4):458–466.
- El-Alfy, M. B.; Abd El-Aty, A. M.; Younis, M. F.; Osman, SH. G. and Gafour, W. A. (2004a). Biological and histopathological changes of rats as affected by feeding on cholesterol—enriched diet containing probiotic Soya Feta like-cheese. Annals of Agric. Sci., Moshtohor, 42(4):1743–1757.
- **Fassati, P. and Principe, L. (1982).** Enzymatic colorimetric method for the determination of triglycerides. Clin. Chem., 28:2077.
- **Gafour, W. A. (2005).** Using of soybean extract to produce some dairy like products. Ph.D Thesis, Fac. Agric., Moshtohor, Benha Univ., Egypt.
- **Gordon, T. M.** (1977). HDL-cholesterol (determination after separation high-density lipoprotein lipid. Amer. J. Med., 62:707.
- **Hatch, F. T. and Lees, R. S. (1968).** Practical methods for plasma lipoprotein analysis. Adv. Lipid Res., 6:1–68.
- **Ibrahim, F. A. S. (2002).** The health potential role of yoghurt and soy-yoghurt containing *bifidobacterium*. Ph.D Thesis, Fac. Agric., Cairo Univ., Egypt.
- Morise, A.; Serougne, C.; Gripois, D.; Blouquit, M. F.; Lutton, C. and Hermier, D. (2004). Effects of dietary alpha-linolenic acid on cholesterol metabolism in male and female hamsters of the LPN strain. J. Nutritional Biochem., 15(1):51–61.
- **National Cholesterol Education program Expert Panel (1994).** Second report of the expert panel on detection, evaluation and treatment of high blood cholesterol in adults. Circulation, 89(3):1333–1445.
- Okazaki, H.; Tazoe, F.; Okazaki, S.; Isoo, N.; Tsukamoto, K. and Sekiya, M. (2006). Increased cholesterol biosynthesis and hypercholesterolemia in mice over expressing squalene synthesis in the liver. J. Lipid Res., 47(9):1950–1958.
- Oluba, O. M.; Adeyemi, O.; Ojieh, G. C.; Aboluwoye, C. O. and Eidangbe, G. O. (2008). Comparative effect of soybean oil and palm oil on serum lipids and some serum enzymes in cholesterol-fed rats. Euro. J. Scientific Res., 23(4):559–566.
- **Putnam, J. and Allshouse, J. (1999).** Food Consumption, Prices and Expenditures, 1970–97. Statistical Bull., 9650 Washington, DC: Food and Rural Economics Division, Economic Research Service, US Department of Agriculture.
- **Reitman, S. and Frankel, S. (1957).** A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Amer. J. Clin. Path., 28:56.
- **Richmond, W. (1973).** Enzymatic colorimetric method for the determination of cholesterol. Clin. Chem., 19:1350.

- **Rozner, S. and Garti, N.** (2006). The activity and absorption relationship of cholesterol and phytosterols. Colloids Surface A: Physicochemical Engineering Aspect, 282–283, 435–456.
- **Sandhya, V. G. and Rajamohan, T. (2008).** Comparative evaluation of the hypolipidemic effects of coconut water and lovastatin in rats fed fat–cholesterol enriched diet. Food &Chemical Toxicology, 46:3586–3592.