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DETERMINE THE EFFICACY OF LACTOFERRIN AND BHA SOFT GENE FORTIFICATION ON EXPERIMENTAL ANIMALS WITH OXIDATIVE STRESS

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Abstract

This study was designed to investigate the effect of lactoferrin and BHA on the total cholesterol level (TC). Total cholesterol, Triglycerides(TG) Triglycerides, High-density lipoprotein cholesterol (HDL-C) High-density lipoprotein cholesterol; low-density lipoprotein cholesterol (LDL-C) Low-density lipoprotein cholesterol, Very low-density lipoprotein cholesterol (VLDL-C) Very low-density lipoprotein cholesterol and the effectiveness of liver enzymes, alt AST and the ALP in adult male rats of the Sparco-Dawley type (Dawley healthy) and oxidative stress patients as an experimental model for this study. The rats were divided into five groups (each with five rats), a healthy control group and a control group affected by oxidative stress, and the three affected groups were fed a standard diet. 50% of the soft cheese is classified as one of three types: (T1) represents soft cheese with no additions. T2 represents lactoferrin fortified soft cheese; T3 represents BHA fortified soft cheese with focus 0.125 mg/L. after the end of the feeding period (28 days), The results showed a significant decrease (p 0.05) in the total cholesterol level of the fed rats. Ton transactions T1 and T2, and previously recorded (138.80 and 116.06 mg/100 ml, respectively, and morally (p 0.05) for the transactions 161.06 and T3, mg/100 ml, compared to the infected rat group by oxidative stress, which reached 159.72 mg/100 ml, In addition to a decrease in the level of triglycerides and low-density lipoproteins in the fed rats in transactions T1 and T2, which registered 76.13 and 54.33 mg/100 ml, respectively, higher for transaction T3, which registered (82.38) mg/100 ml compared to the infected group of rats by oxidative stress, which reached (81.74) mg/100 ml, and a significant increase in the level of high-dens In terms of enzyme activity, the alt and AST The largest significant decrease was in the group of rats fed tons of soft cheese fortified with lactoferrin, which recorded 28.19 and 56.34 international units/liter, compared to the infected control group, which recorded 40.27 and 78.35 international units/liter. It can be concluded that soft cheese fortified with lactoferrin plays a positive role in improving the blood lipid profile in infected individuals by reducing oxidative stress.

Keywords: lactoferrin, BHA, Soft cheese, oxidative stress

Introduction

The nature of each individual's life and his dietary pattern contribute to his overall health condition. It is believed that increased stress, a tense lifestyle, and a poor diet have become features of modern life. It is believed that this has led to an increase in diseases and ill health all over the world. And the free radicals and diseases resulting from an imbalance in the balance of oxidants Antioxidant activity is a hallmark of chronic diseases such as cardiovascular diseases, hepatitis, and cancers. (Llghamiand others,2020). On the other hand, antioxidants and their natural sources have received great attention in recent years, especially after the high incidence of modern diseases

such as heart disease, atherosclerosis, premature aging, and cancer. Recent studies have proven the great link between many diseases and the free radicals formed automatically in our bodies during the natural metabolism of food by breathing oxygen. These active radicals carry out oxidative processes that end up causing disruption and damage to cells and tissues in the body and thus infecting it with diseases, the most dangerous of which is cancer. (Pérez-Torres and others, 2021). It is this antidote, lactoferrin protein. lactoferrin (Lf) that prepares antioxidants and antimicrobials from an animal source and It is a non-specific immune protein characterized by high bioactivity through its ability to bind iron (Bruni and others, 2016). It plays an important role in protecting the body from free radicals through support for enzyme systems P and the SOD and the cat in the galactic excretion for teeth and cows and an increase in their ability to stop oxidation. With sulfuric acids, as these acids enter into the synthesis of the glutathione system, they thus control the level of glutathione in the cell, which reduces the possibility of injury. Atherosclerosis and cancer are both heart diseases (Zhu and Things, 2006). and the butylated hydroxy anisol (BHA). One of the industrial antioxidants added to processed food products, particularly those containing fatty substances, is to protect them from oxidation reactions during preservation and storage, but the health safety of these compounds is still being debated among scientists, as recent studies revealed, for example, that BHA is a carcinogen (Amy and Tran, 2013).

Materials and methods

The source of the samples is Cow's milk was obtained from a private farm in Samarra district/Salah Al-Din Governorate that raises cows. The milk sample was collected in sterile and clean bottles, transferred to the laboratory under cooling conditions, and placed in the refrigerator at a temperature of 25 of, or 25 Cº.

Workers will test the white side (White Side Test) to ensure the safety of milk and the absence of mastitis, and in the narrator's (2005) case, lactoferrin (CoJarrow formulas/USA) was used, while the synthetic antioxidant (BHA) was obtained from a company, Eisen-Golden American.

Chemical tests on milk: The chemical properties of A's milk for cows were examined using a device (the LactoStar Instrument for the Analysis of Milk) of German origin to measure the following ingredients: fat percentage, rate protein, percentage of lactose, mineral element ratio, and percentage of non-greasy solids. and was completed using a device meter and directly according to what was stated in AOAC (2004). And, using phenolphthalein as a guide, I used the method described in Javaidet al. (2009) to measure the acidity of milk samples titrated with sodium hydroxide solution.

Cheese manufacturing: After receiving the milk and examining its quality, the soft cheese was made according to what was needed. Foxet et al. (2017 Soft cheese was divided into three types: Soft cheese without addition is symbolized by T1. T2 represents lactoferrin-fortified soft cheese; T3 represents BHA-fortified soft cheese with a focus of 0.125 mg/L.

This study used experimental animals [25]. An adult male white rat (Rattus norvegicus) of the Sprague-Dawley breed weighing 205-250 g obtained from the University of Florida's College of Veterinary Medicine, Tikrit, they were placed in plastic cages with metal covers and a floor furnished with sawdust. The aspect of cleaning and sterilizing the cages was taken into account, with sawdust being replaced every two days. The animals underwent laboratory conditions for the photocycle, which was divided into 12 light hours and 1 hour of darkness, and the temperature was fixed at 1 hour of darkness, and the temperature was fixed at 225 degrees Celsius. The animals were left for three days to adapt to the new conditions and ensure that they were free of diseases. They were given food and water continuously and in sufficient quantities throughout breeding and puberty. 28day.

Prepare food Weighted: Prepared Weighted food calculations What does the National Academy of Sciences/National Research Council (NAS/NRC) (2002) state about me (158.5 casein/kg, 100g glucose/kg, 50g cellulose/kg, 100g corn oil/kg, 5g vitamin mixture/kg, 50g mineral salt mixture/kg, and 536.55g starch/kg)? Distilled water was added to the mixture to form a cohesive dough and form pieces suitable for feeding rats, which were then placed in flat stainless steel pots and dried in an oven at 50 degrees Celsius by a stream of hot air for a while before being packed in poly bags. The linen was kept in the refrigerator at 52 degrees Celsius for the duration of the experiment.

Experiment design: Experimental animals were randomly assigned to five totals within each group made up of five animals. It was completed by feeding the animals free-balanced food and fortified cheese for a period of 28 days, as follows:

1. The first group (negative control group): These animals were left intact and fed on a standard diet only throughout the duration of the experiment.

2: The second group (positive control group) was fed a standard diet with the addition of hydrogen peroxide H2O2 at a concentration of 5% in water, and it caused stress throughout the duration of the experiment.

3. The third group, T1, the group affected by oxidative stress and nutrients, consumed 50% of the standard mixture and 50% of regular soft cheese throughout the duration of the experiment.

4. The fourth group, T2, the group affected by oxidative stress and nutrients, was fed a 50% diet plus 50% of soft cheese fortified with lactoferrin (20 mg/L) throughout the duration of the experiment.

5. The fifth group, T3, the group affected by oxidative stress and nutrients, was fed a 50% diet plus 50% soft cheese fortified with BHA at a concentration of 0.125 mg/LL throughout the duration of the experiment (Lafteh and Dosh, 2017).

Biochemical tests: After the end of the experiment, the rats were prevented from eating for 12 hours. The animals were then anesthetized with chloroform, and blood was drawn directly from the heart and placed in test tubes. contain a substance (EDTA) and were left for about a quarter of an hour in a 37 °C water bath, after which the serum was obtained by centrifuge at a speed of 3000 rpm for 15 minutes and kept at a temperature of -18 °C until the special biochemical tests, which include measuring the level of cholesterol, triglycerides, high-density lipoproteins (HDL-C), low-density lipoproteins (LDL and Faas et al., 2002).

Statistical analysis

The results of the experiments were analyzed using the general linear model (Linear Model General) within the ready-made statistical program (SAS, 2012). To study the effect of factors according to a complete randomized design (CRD) as conducted by Duncan (1955), to determine the significance of the differences What's wrong with me? Averages of the factors affecting the traits studied at the level (0.05).

Results and discussion

Specifications of the raw milk used in the experiment: Fresh raw cow's milk of good quality was used in the manufacture of soft cheese, and its main components were analyzed using an instrument called the LactoStar Milk Testo. The table shows (the averages of the percentages of the chemical components were within the normal limits of milk, as these results agree with what Al-Jubouri found in 2018), where he found the percentages of moisture, total solids, protein, fat, lactose, and pH at 87.81, 8.90, 3.40, 3.25, 4.75, and 6.64, respectively.

Table (1)modified Pedigree Ingredients the milk user in industry soft cheese

specific density	pH	Acidity (as lactic acid) %	Mineral elements %	lactose%	fat%	protein %	% of total solids	Humidity %	**the ingredients**
1,032	6.8	0.16	0.74	4.83	3.48	3.64	12.65	87.35	**The ratioCentennial**

-Numbers in schedule she modified Three replicates.

Effect of different treatments on blood lipid profile: notes from the table (2) The concentration of cholesterol, triglycerides, low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) was about mg/100 ml in the blood serum of male rats with oxidative stress, while its value decreased when fed with regular soft cheese. The above variables are 138.80, 76.13, 78.83, and 15.22, in that order. It is noted from the results that the fortification of cheese with lactoferrin (T2) led to a significant decrease in the values (116.06, 54.33, 56.86, 10.86). For the above variables in a row, it is noted from the results that fortifying cheese with BHA (T3) led to a significant increase in the values 161.06, 82.38, 118.43, and 16.47 for the above variables, respectively, while it is

noted from Table 2 that the concentration of high-density lipoprotein (HDL) showed a significant decrease. 26.21,26.16to control the infected andT3 There was a significant difference in the values 44.75 and 48.34 for straight transactions T1 and T2.

Table 2: Effect of different treatments on the concentration of very low-density lipoprotein cholesterol (mg/100 ml) in the blood serum of healthy and oxidatively stressed male rats.

treatment	Very low density lipoproteinsvldl mg \100 ml	Low density lipoproteins LDL mg/100ml	High density lipoproteins HDL mg\100 ml	TriglycerideTG mg \100 ml	total cholesterol mg \100 ml
proper control	10.17	**33.74**	**43.15**	**50.89**	**87.06**
infected control	16.34	**117.17**	**26.21**	**81.74**	**159.72**
T1	15.22	**78.83**	**44.75**	**76.13**	**138.80**
T2	10.86	**56.86**	**48.34**	**54.33**	**116.06**
T3	16.47	**118.43**	**26.16**	**82.38**	**161.06**
LSD value	2.958*	**5.753***	**4.894***	**4,840***	**6.923***
. * (P<0.05).					

The numbers in the table represent the mean values ± the standard deviation.

Different letters in one column indicate significant differences ($p<0.05$) among the study groups.

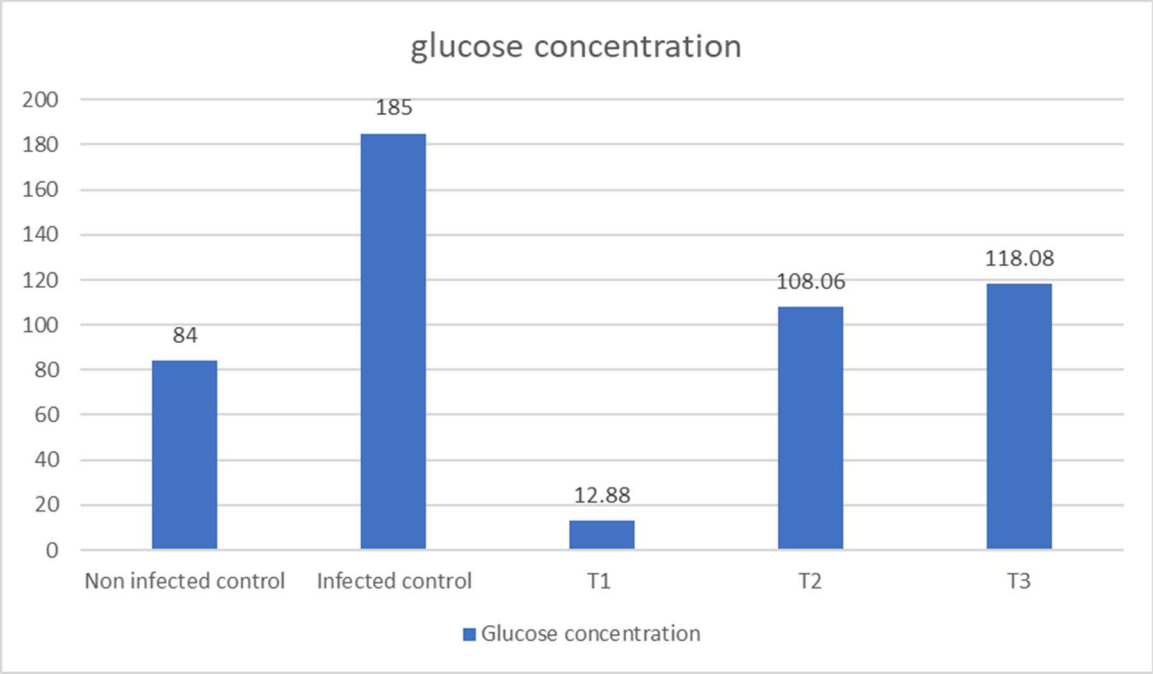
The findings of this study agreed with those of Al-Fahdawi (2018). whichatistics.Al-Fahdawi found (2018) Which indicated a significant increase in the blood lipid profile of rats with oxidative stress, as that stress accelerates the process of decomposing fats and unsaturated fatty acids, and it leads to a decrease in insulin due to the significant damage caused by oxidative stress to the pancreatic beta cells, which leads to a decrease in the activity of the lipoproteinresponsibleyme. Lipoprotein lipase is Responsible fHamdani, 2020dowandf triglycerides (Al-Hamdani,2020). And that the active oxygencalled triglycerideolipase,awhichte an enzyme Triglyceride lipase Which breaks down triglycerides and causes an increase in lipid metabolism and an increasSawa, 2016r levels in the blood serum (Sawa,2016). While treatment with a soft gene fortified morale,actoferrin led to a decrease in morale the decrease in blood lipids was due to the ability of lactoferrin to hinder the aggregation of cholesterol and regulate its levels in the blood. The results were coet al. (2004t andhSamurai (2018nd. Takeuchi al(2004) And samurai(2018) indicated a decrease in blood lipid profile in groups of rats8 were dosed with lactoferrin for time8 weeks, and it is noted from the results that there was a significant increase in the blood lipi. T3 is thought to be the cause of an increase in enzyme activity (cholesterol

acyl transferase) in the intestine, which stimulates the absence of cholesterol. thetine, which stimulates the absence The triggering hormone insulin(2018). It is evident from the results of the analysisstatistic There are significant differences at the level of 0.05 between the treatments for the infected control and T2. For the treatment of the sound control, the group of rats showed that T2 outperformed the rest of the group in lowering blood lipid levels.

Effect of different treatments on blood sugar level

Figure 1 shows a significant increase in the percentage of glucose (85 mg/100 ml) and, on a moral level, P 0.05 in the serum of infected rats treated with hydrogen peroxide (H2O2). In comparison to the healthy control group (mg/100 ml), the blood sugar level (185, 84), and the glycemia of the coefficients T1, T2, and T3 (12.88, 108.06, and 118.08) in succession with the infected control group, we notice significant differences and a decrease in the values of the different coefficients, and note the statistical analysis shows the superiority of the treatment. T2 In adjusting the level of glucose in the blood during the treatment, T3 was the closest to a proper treatment.

Figure (1): Effect of different treatments on glucose concentration (mg/100ml) in serum Blood of healthy and infected rats by oxidative stress



Serum glucose levels in rats exposed to hydrooxygen levels (hyperoxia).gen peroxide oxidative stress H2O2 increased significantly (P 0.05). with drinking water for 30 days and agreed withthe results of Al-Fahdawi, (2018). The cause of high blood sugar is high oxygenhyperoxia which causes an increase in active oxygen species that attack islet cells of Langerhans type beta-cells that secrete insulin, causing a defect in their functions (Al-Hamdani, 2020), thereby disrupting insulin secretion, stopping glucose dissolution and stimulating glucose formation and glycogen breakdown (Peh and others, 2016). Figure 1 shows that there is a drop in morale (P 0.05) in the transaction. T2 The results agreed with what Moreno et al. (2009) found. After demonstrating that there was an increase in the ability of the lactoferrin-treated group to dispose of blood sugar, this

was attributed to an increase in cell sensitivity to the hormone insulin, as lactoferrin works to increase the factor responsible for phosphorylation of glucose (172 Thr) AMPK. And then facilitating the task of entering it into the cell, preventing its exit again, and reducing its levels in the blood as well as controlling the level of fat accumulation that causes obesity by controlling the levels of enzymes in liver cells responsible for its synthesis, such as glycerol-3-phosphate acyltransferase. From the results of the statistical analysis, it is clear that there are significant differences between the treatment and the rest of the transactions if the transaction is superior in adjusting the glucose level to normal limits.

Effect of different treatments on the concentration of liver enzymes

Table (3) shows that there was a significant increase (P 0.05). In a group of infected control rats exposed to oxidative stress by hydrogen peroxide, liver enzymes alt, AST, and ALP had values of 40.27, 78.35, and 109.45 IU/L, respectively. Straight, its value decreased when fed with regular soft cheese (T1) and inform 35.89, 66.95, and 103.45 (IU/L), respectively. It is noted from the results that the fortification of cheese with lactoferrin (T2) led to a significant decrease in the values 28.19, 56.34, and 97.39 (IU/L). For the above variables in a row, it is noted from the results that fortifying cheese with BHA (T3) led to a significant increase in the values 42.53, 80.48, and 111.27 (IU/L) for the above variables, respectively.

Table 3: The effect of various treatments on the efficacy of liver enzymes (IU/L) in the serum of healthy and infected male rats after oxidative stress exposure.

treatment	ALP (IU/L)	AST (IU/L)	ALT (IU/L)
proper control	92.53 ±1.56d	54.48 ±2.05a	26.21 ±0.85e
infected control	109.45 ±2.02c	78.35 ±1.27e	40.27 ±1.14b
T1	103.46 ±2.25b	66.99 ±1.76+d	35.39 ±1.03b
T2	97.39 ±0.57e	56.34 ±1.85c	28.19 ±0.95c
T3	111.27 ±2.30a	80.48 ±1.97b	42.53 ±1.78a

The different letters in the same column indicate that there are significant differences between the study groups.

$p<0.05$ The numbers in the table represent the mean values ± the standard deviation.

The results agreed with what was found by Preacher (2015), where he indicated that there was a significant increase in the concentration of liver enzymes in the blood serum of rats affected by oxidative stress, and the reason for this is attributed to destroying the cell membranes of the liver cells, increasing the rate of lipid peroxidation, and raising the level of ferritin protein concentrations (Ferritin) in the blood serum as a result of cirrhosis of the liver, and these protein

substances pass into the bloodstream and impair the removal of these substances from the blood (Al-Mohammadi, 2013). While the treatment was performed with soft cheese fortified with lactoferrin T2, this study showed a significant decrease in the concentration of liver enzymes in the blood serum of infected rats. Rizk, and others (2018) The use of lactoferrin in the treatment of hepatitis led to a significant decrease in liver enzymes, and the reason for this is attributed to the anti-inflammatory effect of lactoferrin resulting from preventing the activation of liver enzymes due to the effect of its antioxidants (Cohen-Naftali, 2011). It is also noted from the results that the soft cheese fortified with BHA led to a significant increase in liver enzymes ALP, ALT, and AST, which matched what was indicated by Aml (2013). And, as mentioned above, Varuna et al. (2014) reported an increase in the effectiveness value of silver enzymes, The reason for the rise in enzymes is due to the occurrence of severe damage to the liver and thus injury to the liver cells, which causes enzymes to be released into the blood and their levels to rise (Lambert and Hull, 1996). The destruction of liver cell membranes due to increased lipid peroxidation and an increased concentration of free radicals causes the leakage of enzymes into the blood serum (Al-Hazza et al., 2008).

Reference

1. **Al-Fahdawi, Othman Eid Saleh (2018).** Comparison of the effect of aqueous extract of cuminum seeds with vitamin E on some physiological variables and reproductive system fertility in rats exposed to oxidative stress. University of Tikrit.
2. **Al-Hamdani, Hanan Majed Mohammed, (2020),** Effect of Cucurbita pepo Pumpkin Extract on Some Hematology, Biochemical and Histological Parameters in Rats Exposed to Oxidative Stress, Master's Thesis, College of Education for Girls, University of Tikrit.
3. **Al-Hazza, I. M., Ibrahim, S. A., Bashandy, S. A. and Alshehry, S. A. (2008).** Protective effect of vitamin B_6 against carbon tetrachloride induced hepatotoxicity in male rats: Effect on Liver enzymes, glucose, total protein and insulin hormone. Saudi J. Biol. Sci., 15: 75-83.
4. **Al-Jubouri, Orouba Bahjat Shehab, (2018),** Evaluation of the physicochemical and bacteriological characteristics of yakut and yogurt and determining their effectiveness in some physiological parameters of laboratory rats, Master's Thesis, Faculty of Agriculture, University of Tikrit.
5. **Al-Mohammadi, Qusai Nouri Radam. (2013).** Dr. RA Physiology, Biochemical and Tissue on the protective effect of olive oil and lemongrass extract in the liver and brain of local rabbits exposed to oxidative stress. Thesis of Dr. A.R., Faculty of Education for Pure Sciences, University of Tikrit.
6. **Al-Qazzaz, Ahmed Salah El-Din Mohieldin, (2018),** Estimation of Some Industrial Antioxidants in Food and Study of Their Biological Effects in Male White Rats, Master's Thesis, Faculty of Agriculture, University of Tikrit.
7. **Al-Samarrai, Muhammad Subhi Khattab, (2018),** Isolation, purification and characterization of lactoferrin from whey and its use in the preservation of raw milk and in the manufacture of therapeutic milk, Master Thesis, College of Agriculture, University of Baghdad

8. **Aml, F. E. (2013).** Effects of butylated hydroxytoluene and butylated hydroxyanisole against hepatotoxicity induced by carbon tetrachloride in rats. World Applied Sci. J., 22: 63-69.
9. **Amy, V. T. (2013).** Do bha and bht induce morphological changes and dna double strand breaks in *Schizosaccharomyces pombe*. A Thesis of Claremont McKenna, Pitzer and Scripps Colleges, Claremont University, U. S. A.
10. **AoAC,(2006).** Official Methods of Analysis ,14th Ed. Association of Official Analytical Chemists , Washington , D.C.
11. **Bruni, N.; Capucchio, M. T.; Biasibetti, E.; Pessione, E.; Cirrincione S. and Giraudo, L. (2016).** Antimicrobial activity of lactoferrin-related peptides and applications in human and veterinary medicine. Molecules 21, 752. 10.3390/molecules21060752.
12. **Cohen-Naftaly, M., and Friedman, S. L. (2011)** Current status of novel antifibrotic therapies in patients with chronic liver disease. *Therap. Adv. Gastroenterol.* **4**, 391– 417.
13. **Duncan, D.B. (1955).** Multiple range and multiple "F" test. Biometric, 11:1-42.
14. **Faas, F. H.; Earlewine, A.; Smith, G. and Simmons, D. L. (2002)**. How should low-density lipoprotein cholesterol concentration be determined? J. Fam. Pract. 51:973-975.
15. **Fox, P. F.; Guinee, T. P.; Cogan, T. M. and McSweeney, P. L. (2017).** Overview of cheese manufacture. In Fundamentals of cheese science (chapter 1). Springer US. pp 11-25.
16. **Khatib, Souad Osama (2015).** Effect of Red Tea Extract on Hematological Parameters and Oxidative Stress in Male White Rabbites, Iraqi Journal of Science, Vol. 56, No. 4, pp. 3370-3357.
17. **Lafta, Shaima Saadi: Dosh, Kifah Saeed (2017).** Study the effect of nutrition on low-fat mozzarella cheese using fat substitutes represented by beta-glucanß and inulin in some health and physiological indicators of white mice. Tikrit University Journal of Sponsored Sciences – Special Issue of the Proceedings of the Sixth Scientific Conference on Agricultural Sciences, March 28-29.
18. **Lambert, J. and Hull, R. (1996)**. Upper gastrointestinal tract disease and probiotics. Asia Pacific J. Clinical Nutr., 5: 31-35.
19. **Llghami, R, Barzegari, A, Mashayekhi, M, R, Letourneur, D, Crepin, M, Pavon-Djavid, G(2020).** The conundrum of dietary antioxidants in cancer chemotherapy. Nutrition Reviews. Issue 1(Volume 78) : Pages 65-76.
20. **Moreno-Navarrete, J.M.; Ortega, F.J.; Bassols, J.; Ricart, W. and Ferna´ndez-Real, J.M.(2009).** Decreased circulating lactoferrin in insulin resistance and altered glucose tolerance as a possible marker of neutrophil dysfunction in type 2 diabetes. J Clin Endocrinol Metab 94: 4036–4044.
21. **National Academy of Science – National Research Council (NAS/NRC). (2002)**. Dietary Allowance. 15th ed. Washington. D.C. National Academy. Press.
22. **Pérez-Torres, I.; Castrejón-Téllez,** V.; Soto, M.E.; Rubio-Ruiz, M.E.; Manzano-Pech, L.; Guarner-Lans, V. Oxidative Stress, Plant Natural Antioxidants, and Obesity. Int. J. Mol. Sci. 2021, 22, 1786.

23. **Rizk, F, H. Sarhan, N,I. Soliman, N,Abd-Elsalam, M. Abd-Elsalam, SH. (2018).** Heat shock protein 47 as indispensable participant in liver fibrosis: possible protective effect of lactoferrin. orcid.org/0000-0003-4366-2218 Departments of Tropical, Faculty of Medicine, Tanta University, Egypt
24. **SAS.(2012).** Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.
25. **Sawa, T. (2016).** Oxidative Stress Regulation by Reactive Cysteine Persulfides in Inflammation. In Chronic Inflammation (pp. 309-316). Springer Japan.
26. **Takeuchi ,T.; Shimizu, H.; Ando, K. and Harada ,E.(2004).** Bovine lactoferrin reduces plasma triacylglycerol and NEFA accompanied by decreased hepatic cholesterol and triacylglycerol contents in rodents. *Br J Nutr*;91:533–538.
27. **The narrator, Zaid Akram Thabet. (2005) .** Isolation and diagnosis of some types of Lactobacillus bacteria capable of reducing cholesterol and introducing them into therapeutic lactoferments. Master's Thesis. College of Agriculture - University of Baghdad.
28. **Tietz, Y., (2005),** Clinical Biochemistry, 6th ed., McGraw –Hill, New York. 825.
29. **Varuna, P., Panicker, S. and George, D. K. (2014).** Toxicity study of butylated hydroxy toluene (BHT) in rats. World J. Pharmacy and Pharmaceutical Sci., 3: 758-763.
30. **Young, D. S.(2000).** Effects of drugs on clinical laboratory tests, 5th ed. AACC press.
31. **Zhu, Y.; Carvey, P.M. and Ling, Z. (2006).** Age-related changes in glutathione and glutathione-related enzymes in rat brain. Brain Res. 1090, 35–44.