Tikrit Journal for Agricultural Sciences (2024) 24 (4): 235-245
DOI: **https://doi.org/10.25130/tjas.24.4.19**

ISSN:1813-1646 (Print); 2664-0597 (Online)
Tikrit Journal for Agricultural Sciences
Journal Homepage: http://www.tjas.org
E-mail: tjas@tu.edu.iq

Level of Lipid Profile and Liver Enzymes Hypercholesterolemic Rats Treated with Actimel Milk

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KEY WORDS:
Actimel milk, hypercholesterolemia, blood lipid profile.

Received: 27/03/2024
Revision: 26/07/2024
Proofreading: 22/09/2024
Accepted: 13/08/2024
Available online: 31/12/2024

ABSTRACT

This study was designed to determine the effect of Actimel milk on the weights of rats and the level of Total cholesterol (TC), Triglycerides (TG), High density lipoprotein cholesterol (HDL-C), Low density lipoprotein cholesterol (LDL-C), Very low density lipoprotein cholesterol (VLDL- C), and the effectiveness of liver enzymes ALT, AST, and ALP in healthy adult male Sprague-Dawley rats suffering from hypercholesterolemia as an experimental model for this study. The rats were distributed into four groups (six rats each): the healthy control (T1), the infected control (T2), the infected and treated with yogurt milk (T3), and the infected and treated with Actimel milk (T4). After the end of the milk feeding period (28 days), the results showed a significant decrease ($p<0.05$) in the weight of the rats and the levels of TC, TG, LDL-C, and VLDL-V, and a significant increase in the HDL-C values of the rats fed the milk of the groups (T3, T4) compared with the infected control group (T2). As for the activity of liver enzymes ALT, AST, and ALP, the largest decrease was in the (T4) group compared to the infected control group (T2). It can be concluded that Actimel milk played a positive role in improving the blood lipid profile in rats suffering from hypercholesterolemia.

مستوى الدهون وإنزيمات الكبد في الجرذان المصابة بفرط كوليستيرول الدم والمعالجة بلبن ألاكتيميل

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الخلاصة

تم تصميم هذه الدراسة لتحديد تأثير حليب Actimel على أوزان الفئران ومستوى الكوليسترول الكلي ، والدهون الثلاثية ، والكوليسترول الدهني عالي الكثافة ، والكوليسترول الدهني منخفض الكثافة ، والكوليسترول الدهني منخفض الكثافة ، وفعالية إنزيمات الكبد في ذكور الفئران الذين يعانون من فرط كوليسترول الدم كنموذج تجريبي لهذه الدراسة. تم توزيع الفئران على خمس مجموعات (ستة فئران لكل منها): السيطرة الموجبة ، والسيطرة المصابة ، والمصابة والمعالجة بحليب الزبادي ، والمصابة والمعالجة بحليب Actimel. بعد انتهاء فترة تغذية الحليب (28 يوما) ، أظهرت النتائج انخفاضا كبيرا في وزن الفئران ومستويات دهون الدم ، وزيادة كبيرة في قيم البروتينات الدهنية عالية الكثافة في الفئران التي تم تغذيتها على الحليب من المجموعة الثالثة والرابعة مقارنة مع المجموعة الضابطة المصابة، أما بالنسبة لنشاط إنزيمات الكبد كان أكبر انخفاض في مجموعة الرابعة مقارنة مع المجموعة الضابطة المصابة . يمكن الاستنتاج أن حليب Actimel لعب دورا إيجابيا في تحسين مستوى الدهون في الدم لدى الفئران التي تعاني من فرط كوليسترول الدم.

الكلمات الافتتاحية: حليب أكتيميل ، فرط كوليسترول الدم ، مستوى الدهون في الدم.

INTROUCTION

There is a positive relationship between some chronic diseases that threaten human safety and health and poor dietary behavior related to eating fats in quality and quantity, due to the prevalence of eating fatty foods containing types of saturated fats, especially the trans fatty acid type Rich in cholesterol, especially animal proteins (Pipoyan *et al.*, 2021) . Numerous studies on humans and animals have proven that increasing the intake of cholesterol and saturated fat causes an increase in oxidative stress, which is defined as a disturbance in the balance between. Antioxidant defenses and free radicals in the tissues, which is one of the factors leading to the development of atherosclerosis, which is one of the cardiovascular diseases (CVD) (Ponnampalam *et al.*, 2022).

The growing human health awareness and the idea of rejecting chemical treatments in view of the known health risks they cause, the idea of relying on natural sources as alternative treatments for or prevention of many diseases, which are almost free of side effects (Chaughule and Barve, 2024). was born, as therapeutic microorganisms are among the most important sources that have occupied It has a wide area in the research department, regardless of its various medical and pharmaceutical specializations in the field of life sciences research and in agricultural fields, especially in the field of food industries. With the acceleration of the pace of scientific discoveries in recent times and the expansion of scientific research spaces based on the gifts of nature, the benefits of therapeutic microorganisms have emerged, which occupy the summit. Lactic acid bacteria, especially the genus Lactobacillus, as recent years have witnessed a widespread trend towards using these types of bacteria in the manufacture and preservation of a number of food products, in addition to their role in improving the flavor and texture of food and prolonging its shelf life (Jassim *et al.*, 2020).

Actimel milk is one of the types of fermented milk that contains Lb.casei and Bifidobacterium bacteria, which contain fat, protein, lactose, and ash at a rate of 3.1, 3.2, 2.98, and 0.81%, respectively (AL-Jashe, 2018). Many studies and scientific research have indicated the ability of fermented milk containing Lactobacillus and Bifidobacterium bacteria to reduce blood sugar, total cholesterol, and fats in culture media, animal blood serum, and humans through their ability to metabolize cholesterol while food passes through the intestine, making it non-absorbable. Or its ability to dissociate bile salts, which prevents its absorption in the enterohepatic circulation, thus increasing the demand for cholesterol as precursors for the synthesis of bile salts (Shihab et al., 2023). The current study aimed to investigate the effect of Actimel milk on blood lipid profile and liver enzymes in rats with hypercholesterolemia.

MATERIALS AND METHODS

Actimel milk: Actimel milk samples were obtained from local markets in the city of Erbil/Iraq and kept in the refrigerator at 5±2°C until chemical and biological tests were conducted.

Yogurt milk: A starter consisting of Streptococcus thermophilus and Lactobacillus bulgaricus was used, which was obtained in dried form from the College of Agriculture / University of Baghdad. The starter was activated before use three times in a row. The milk was heated to a temperature of 85-90°C/15 minutes and then cooled to 45°C, adding 3% starter culture, then packing in plastic containers and incubating at 42°C until yoghurt is formed (Tamime and Robinson, 1999). Cool the resulting yogurt at a temperature of 5±2°C until the required analyzes are performed.

Physicochemical tests for milk: The percentage of moisture, carbohydrates, ash and total acidity in milk was estimated according to what was stated in A.O.A.C (2008). As for protein, it was estimated according to the method mentioned in (Ling, 2008), and the percentage of fat was estimated using a German-made Kerber device, according to Min and Ellefson (2010). While the pH was estimated by placing the sensor of a pH meter, model 211, type HANNA, of Romanian origin, directly in the milk sample (Hool et al., 2004).

Experimental animals: 24 adult male white rats (Rattus norvegicus) aged 2-3 months were used, which were obtained from the College of Veterinary Medicine / Tikrit University and placed in metal cages with metal covers. The animals were subjected to laboratory conditions of a light cycle that was divided into (12 One hour of light and (12) hours of darkness, and the temperature was fixed at (2±25) degrees Celsius. The animals were left for three days to adapt to the new conditions and to ensure that they were free of diseases. They were given food and water continuously (adlibitum) in sufficient quantities throughout the rearing period.

Weighed food: Weighed food was prepared according to what was stated in the National Academy of Science/National Research Council (NAS/NRC, 2002) to contain (158.5 gm casein/kg, 100 gm glucose/kg, 50 gm cellulose/kg, 100 gm corn oil/ kg, 5 gm vitamin mixture/kg, 50 gm mineral salt mixture/kg, and 536.5 gm starch/kg). Distilled water was added to the mixture to make a cohesive dough and to form pieces suitable for feeding the rats. Then it was placed in flat stainless steel utensils and dried in an oven at a temperature of 50 °C using a hot air flow until complete drying. Then it was packed in polyethylene bags and stored in the refrigerator at a temperature 5°C±2 throughout the experiment.

Preparation of the high-fat diet: The high-fat diet was prepared based on what was mentioned by De Meijer and others (2010) to contain fat, protein, carbohydrates, beef tallow, cholesterol and a mixture of vitamins and minerals in the amount of 58, 25, 17, 13, 1 and 0.6. % respectively.

Experiment design: Experimental animals were randomly distributed into four groups, each group consisting of six animals. They were fed a high-fat diet for four weeks, with the exception of the first group, which was fed a weighed diet only. After the end of the 28-day infection period, the experimental animals were fed fermented milk, and the doses were given at a dose rate of 12 hours(aL-Jobouri,2018) , as follows:

1. The first group: (Negative control group): These animals were left intact and were not given milk, while continuing to give water and weighted food throughout the duration of the experiment (T1).
2. The second group: (positive control group): They were fed a high-fat diet for the duration of the experiment (T2.
3. The third group: was fed a high-fat diet and dosed with 1 ml of yogurt milk for the duration of the experiment (T3).
4. The fourth group: fed a high-fat diet and dosed with 1 ml of Actimel milk for the duration of the experiment (T4).

Biochemical tests: After the end of the experiment period, the animals were weighed, then the rats were deprived of food for approximately 12 hours. The animals were then anesthetized with chloroform, then blood was drawn directly from the heart and placed in test tubes that did not contain the substance (EDTA) and left for approximately a quarter of an hour in a bath. in aqueous temperature at 37°C, after which the serum was obtained using a centrifuge at a speed of 3000 rpm for 15 minutes and stored at a temperature of (-18)°C until special biochemical tests were performed, which include measuring the level of cholesterol, triglycerides, and high-density lipoproteins. (HDL-C), low-density lipoproteins (LDL-C), very low-density lipoproteins (VLDL-C), alline aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in sera. animals according to the methods used before (Tietz, 2005).

Statistical analysis: The results of the experiments were analyzed using the General Linear Model within the ready-made statistical program (SAS, 2004) to study the effect of the factors according to the complete random design (CRD). The Duncan test (Duncan, 1955) was also conducted to determine the significance of the differences between the averages of the influencing factors on the studied characteristics at the level of (0.05).

RESULTS AND DISCUSSION

Table 1 shows the chemical composition of yogurt and actimel milk used in the study. It is noted from the table that the percentage of moisture in the milk reached 87.09 and 85.74%, respectively, the percentage of protein reached 3.54 and 4.86%, respectively, and the percentage of fat was 3.38 and 3.68% for yogurt and full milk, respectively. It is clear that the percentage of ash was 0.71 and 0.74%, respectively, while we find that the pH value of the fermented milk was recorded at 4.53 and 4.20, respectively.

Table 1 Physicochemical properties of Actimel milk

Treatments	%						pH
	Moisture	Protein	Fat	Carbohydrates	Ash	Total acidity	
yogurt milk	87.09 1.14±	3.54 0.45±	3.38 0.09±	5.28 0.25±	0.71 0.05±	0.85 0.03±	4.53 0.07±
Actimel milk	85.74 ± 1.17	4.86 ± 0.28	3.68 ± 0.44	4.98 ± 0.39	0.74 ± 0.04	0.91 ± 0.02	4.20 ± 0.73

-The numbers in the table are average for three replicates.

These results are consistent with the findings of AL-Jobouri (2018), who indicated that the percentage of moisture, protein, fat, carbohydrates, ash, and total acidity value of yogurt milk was at 87.15, 3.48, 3.41, 5.26, and 0.70. , 0.84%, respectively, and the pH value is 4.50. These results are also consistent with what was indicated by AL-Jashe (2018), which showed that the percentages of the components of Actimel milk were 85.89, 4.76, 3.78, 4.85, and 0.72% for moisture, protein, fat, carbohydrates, and ash, respectively. . It was also found that the total acidity value was 0.90% and the pH of Actimel milk was 4.22.

The effect of different treatments on weight: Table 1 shows the effect of feeding a standard diet (T1), a high-fat diet (T2), and a high-fat diet with the rats dosed with 2 ml of yogurt milk daily (T3) and a high-fat diet with the rats dosed with 2 ml. of Actimyl milk (T4) on the rate of daily gain and final weight gain among groups of experimental rats after 28 days of feeding and dosing. It was clear from the results that the daily weight gain of individuals in the group of rats that were fed a standard diet amounted to (0.086) gm/day and the final weight gain was (2.42) grams. The table also shows that the highest daily increase appeared in rats fed a high-fat diet, where the average daily increase reached (0.561) grams/day and the final weight gain was (15.73) grams. As for the average weight gain The daily rate of increase in members of the group of rats fed a high-fat diet and dosed with yogurt milk amounted to (0) gm/day, and the final decrease reached (-9.64) grams, while in rats fed a high-fat diet and dosed with Actimel milk, the daily rate of increase reached (0) gm/day and the final weight loss reached (-12.33) gm.

Table (2) Effect of different treatments on rat weights (g)

Treatment	Body weight (g)		Body weight gain	Average daily increase in body weight (g)
	Initial body weight	Final body weight		

T1	208.54 ±2.14^{b}	210.96 ±1.35^{d}	2.42	0.086
T2	229.03 ±2.44^{a}	244.76 ±2.81^{a}	15.73	0.561
T3	227.85 ±1.76^{a}	218.21 ±2.29^{b}	-9.64	0
T4	227.49 ±2.47^{a}	215.16 ±1.95^{c}	-12.33	0

The numbers in the table express average values ± standard deviation.
Different letters in the same column indicate the presence of significant differences ($p<0.05$) between the study groups.
T1 = healthy control, T2 = infected control, T3 = infected and treated with yogurt, T4 = infected and treated with Actimel.

The average weight gain in rats treated with cholesterol is the result of an increase in the concentration of triglycerides and cholesterol, in addition to an increase in low-density lipoproteins. This increase is considered natural as a result of feeding the animals a high-calorie diet (Rosnah et al., 2022). This result is also consistent with The findings of Abdullah *et al.*, (2022) when he treated rats with a diet containing cholesterol were attributed to the fact that treatment with cholesterol may have caused an imbalance in the metabolism and absorption of fat through its effect on the mechanism of fat oxidation and thus led to the accumulation of fat in specific areas of the body. These results are also consistent with what was found by Karimi et al. (2015) who indicated the effectiveness of fermented milk containing probiotic bacteria in reducing the weight of experimental animals. The results of our study also agreed with what was reported by Kobyliak *et al.*, (2016), who indicated that feeding the rats a high-cholesterol diet and then dosing them with Actimel milk led to a significant decrease in the weight of the rats. This is due to their ability to improve the metabolism process and restore the equality of the distribution of fatty acids, in addition to improving movement. The intestines, thus enhancing the efficiency of the digestive process and the digestive system in general. It is consistent with what was stated by Lee and Salminen (2009), as they conducted several experiments on rats and humans, and the results of their study showed that probiotics led to a reduction in fat mass and body weight, which led to a reduction in the hormone leptin, as it is directly linked to indicators of fat and body mass.

The effect of different treatments on the blood fat profile: The results of the biochemical analysis shown in Table 3 showed that there were significant differences ($P\leq0.05$) in the biochemical tests in the experimental groups. The cholesterol level in the group of infected animals increased to 211.39 (mg/dL) compared to what it was. In the healthy control group, it reached 104.26 (mg/dL). While treatment with yogurt and Actimel led to a significant decrease in cholesterol levels, reaching 167.15 and 143.42 (mg/dL), respectively. It is also noted from the table that there was a significant increase in the concentration of triglycerides for the group of infected animals, which amounted to 148.57 (mg/dL) compared to the healthy control group, which amounted to 82.52 (mg/dL), while it was observed that there was a significant decrease in the level of triglycerides in the blood serum of animals dosed with milk. Yogurt and Actimel recorded 136.58 and 125.43 (mg/dL), respectively. The results also showed a significant decrease at the probability level ($P\leq0.05$) in the level of high-density lipoproteins for the infected group, reaching 26.27 (mg/dL) compared to the healthy control group, which amounted to 40.39 (mg/dL), while it was observed that HDL- C in the groups of infected animals treated with yogurt and ketmil milk increased significantly, reaching 31.98 and 37.62 (mg/dL), respectively. It is also noted from the table that there was a significant increase in the level of low-density lipoproteins for the group of infected animals, which amounted to 155.41 (mg/dL), compared to the healthy control group, which amounted to 47.37 (mg/dL), while a significant decrease was observed in the level of LDL-C in blood serum. Animals dosed with yogurt and Actimel recorded 107.86 and 80.72 (mg/dL), respectively.

Table (3): Effect of Actimel milk on blood lipid profile

Treatment	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
T1	104.26[c] ±2.43	82.52[c] ±2.16	40.39[a] ±1.01	16.50[c] ±0.29	47.37[c] ±1.14
T2	211.39[a] ±2.91	148.57[a] ±3.31	26.27[c] ±1.07	29.71[a] ±0.57	155.41[a] ±2.36
T3	167.15[c] ±2.18	136.58[c] ±3.65	31.98[c] ±1.39	27.31[c] ±0.43	107.86[c] ±1.02
T4	143.42[b] ±2.50	125.43[b] ±2.11	37.62[b] ±1.15	25.08[b] ±0.88	80.72[b] ±1.67

The numbers in the table express average values ± standard deviation.
Different letters in the same column indicate the presence of significant differences ($p<0.05$) between the study groups.
T1 = healthy control, T2 = infected control, T3 = infected and treated with yogurt, T4 = infected and treated with Actimel.

Increasing the dietary content of cholesterol and its absorption by the intestines stimulates the liver to produce large quantities of LDL and VLDL particles to transport triglycerides and cholesterol to the body's tissues via the bloodstream. Feingold (2021) indicated that a diet rich in cholesterol and saturated fats increases the level of apoB mRNA in the liver, which is involved in Formation of the LDL particle. Increasing the fat content of the body tissues reduces the sensitivity of the receptors for LDL lipoprotein particles in the liver, limits the gene expression of the LDL receptors, and reduces the activity of the enzyme Lipoprotein Lipase and Hepatic Lipase. Thus, the level of LDL and VLDL in the blood plasma increases, and stimulates the liver to produce particles. High-density lipoproteins (HDL) transport cholesterol from the blood plasma to the liver in the process of reverse cholesterol transport. However, the maintenance of the level of VLDL, LDL, and cholesterol at a high level despite the high level of HDL may indicate weak activity of the enzyme Lecithin cholesterol acyltransferase (LCAT), which transports cholesterol from the surface The HDL molecule returns to its center to continue taking another cholesterol molecule, as well as a decrease in the production of Cholesteryl ester transfer protein (CEPT), which transports triglycerides to the HDL molecule (Ossoli *et al.*, 2016). The results showed that dosing rats with high cholesterol with yogurt milk led to a significant decrease in blood lipid profile, and this agrees with what was found by aL-Jobouri (2018), who indicated that feeding rats with high cholesterol with 1 ml of yogurt milk daily led to a significant decrease in Concentration of blood fat profile. The reason for this was attributed to the role of fermented milk containing probiotic bacteria in reducing blood fats in experimental animals suffering from hyperlipidemia. Feeding rats with Actimel milk reduces blood fats because it contains probiotics that have the ability to lower blood fats and raise the level of HDL in blood serum. This is due to the ability of bacteria to reduce cholesterol for several reasons, including the ability of probiotics to produce enzymes that break down fat to benefit from it as a source. For carbon, this is what was confirmed by AL-Jashe (2018), who explained the ability of Actimel milk to reduce cholesterol and that is due to the role of probiotics in converting it into bile acids via Bile Salt Hydrolase and the ability of the enhancer to bind cholesterol to the small intestine. The reason for the ability of bacteria to reduce blood fats may be due to the fact that Bacteria can incorporate cholesterol into the cell membranes of the probiotic while it is inside the digestive tract (Karimi et al., 2015).

The effect of different treatments on the level of liver enzymes: Table 4 shows the effect of feeding a standard diet, a high-cholesterol diet, and a diet rich in cholesterol, while dosing the rats daily with 2 ml of yogurt milk and Actimel milk on the activity of liver enzymes in blood serum. It is clear from the table that the highest concentration of ALT level was recorded in The serum of the group of infected control rats (T2) reached 51.08 units/liter, compared to the control sample (T1), which recorded 28.13 units/liter, while its concentration in the groups of infected rats dosed with yogurt milk (T3) and Actimel milk (T4) reached (39.48 and 35.81).) units/liter, respectively. As for the AST enzyme, the highest concentration was in the serum of the (T2) group, as its value reached 105.17 units/liter, compared to the healthy control group, which recorded 79.41 units/liter. It is clear that there are significant differences at the probability level ($P \leq 0.05$), as for dosing with treatments. T3 and T4 for the previous groups decreased to (88.21 and 82.95) units/liter, respectively. The highest value of the ALP enzyme was recorded in the serum of the infected control group, reaching 274.85 units/liter compared to the control group, while its concentration in the infected groups and those dosed with yogurt and Actimel reached (270.69 and 267.09) units/liter, respectively.

Table (4): The effect of Actimel milk on liver enzymes

Treatment	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
T1	28.13 $\pm 0.77^{d}$	79.41 $\pm 1.87^{d}$	265.58 $\pm 3.89^{d}$
T2	51.08 $\pm 0.91^{a}$	105.17 $\pm 1.93^{a}$	274.85 $\pm 2.19^{a}$
T3	39.48 $\pm 0.62^{b}$	88.21 $\pm 1.74^{b}$	270.69 $\pm 2.35^{b}$
T4	35.81 $\pm 0.41^{c}$	82.95 $\pm 0.94^{c}$	267.09 $\pm 2.11^{c}$

The numbers in the table express average values ± standard deviation.
Different letters in the same column indicate the presence of significant differences ($p<0.05$) between the study groups.
T1 = healthy control, T2 = infected control, T3 = infected and treated with yogurt, T4 = infected and treated with Actimel.

The results regarding the increase in liver enzymes in the group of animals suffering from hyperlipidemia agreed with Emamat et al. (2018), as the high content of the diet in total fat led to damage to the liver tissue due to the health problems caused by the fat in terms of high blood pressure and impeding its smooth flow. Within various organs, including liver tissue, which caused an increase in liver enzymes in the blood serum as a result of their exit from the cells. Mohamed and others (2018) indicated that feeding rats a high-calorie diet led to an increase in the percentage of saturated fatty acids in the liver, which may reflect liver injury from By raising the level of liver enzymes. These results are also consistent with the findings of aL-Jobouri (2018), who found a significant decrease in the values of liver enzymes (ALP, AST, ALT) for rats with high fat and treated with yogurt milk. He attributed the reason for this decrease to the role of lactic acid bacteria in improving metabolic indicators. In the liver and then improving its functions by improving its metabolic processes. AL-Jashe (2018) also pointed out that lactic acid bacteria such as *Lb. casei* has the ability to inhibit some compounds responsible for oxidative processes in the body, such as tert-butyl hydroperoxide (t-BHP), which causes damage to liver cells. Therefore, consuming food products containing these bacteria provides protection for liver cells. The reason for this decrease is also attributed to the role of Lactobacillus bacteria in improving metabolic indicators in the liver and thus improving its functions by improving its metabolic processes (Minelli et al., 2004). The results of the groups of infected rats dosed with therapeutic milk were within normal limits for the levels of these two enzymes in the blood serum, which are considered sensitive indicators of many diseases, including heart disease, liver damage, inflammation, or liver enlargement, as their secretion increases when there is any defect in liver function (Johnson- Delaney, 1996).

CONCLUSION

The Actimel group showed significant reductions in body weight, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C), alongside increased high-density lipoprotein cholesterol (HDL-C) levels. Additionally, liver enzyme activities (ALT, AST, ALP) were notably lower in the Actimel-treated group compared to the infected control group. These findings suggest that Actimel milk effectively improves lipid profiles and liver function in hypercholesterolemic conditions, indicating its potential as a beneficial dietary intervention for cardiovascular health. Further research could explore the mechanisms behind these effects and assess long-term benefits.

CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the staff of the Department of Food Sciences, College of Agriculture, Tikrit University for their technical and general support.

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