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Physiological Effects of Zinc Nanoparticles, Lactoferrin, and Neptomycin on Blood types and Lipid Profiles of Albino Rats

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Abstract:

The study was conducted in the laboratories of the Department of Food Sciences - College of Agriculture and in the animal house of the College of Veterinary Medicine at Tikrit University for the period from 1/2/2021 to 15/3/2022. The aim of this study was to manufacture edible whey protein membranes with concentrations of 10%, as well as fortify these membranes with zinc nanoparticles, natamycin and lactoferrin with different concentrations and interactions. The results showed that feeding rats with zinc nanoparticles (ZnNPs) at a concentration of 0.25 mg, natamycin at a concentration of 50 mg, and lactoferrin at a concentration of 60 mg, the erythrocytes (RBCs) had a significant difference ($p < 0.05$), as they were between 5.20-5.35 /mm 610, while the platelets were PLT. 8T: is the highest value, reaching 229 mg/dl. As for the levels of hemoglobin in the blood, it increased significantly ($p<0.05$) for all samples, as it was between 12.20-13.60 mg/dl. Also, the results of white blood cells showed a significant decrease ($p<0.05$) in the values of their cell numbers, as it was between 7.18 – 9.70 / mm 610. Feeding rats with these wraps and their treatments led to a decrease in the values of triglycerides, cholesterol, LDL and VLDL, while the values of HDL were high.

Introduction:

Whey is a by-product of the cheese industry, and it is a transparent liquid part of milk that contains whey proteins, lactose, and minerals. Whey proteins are isolated with the help of various techniques in order to organize the disposal of untreated whey and to identify its nutritional and functional properties. Therefore, whey has been used in many food industries as well as its use As a source of protein and dietary supplement (Ahmad et al., 2019). In recent years, the food packaging industry has also received extensive attention from researchers in the field of food safety and environmental protection, with a focus on how to create edible and biodegradable packaging materials (Baswal et al., 2020), in addition to its excellent mechanical properties, and has the ability to retain moisture, gases, and odors. In addition to the inhibitory activity against microorganisms that cause food spoilage, which caused the extension of the life of these storage materials and the non-use of harmful preservatives and traditional packaging materials, which scientific research has proven to have negative effects on humans and their health (Costa et al., 2018), which may interact With food and its components to produce toxic and pathogenic compounds that have negative effects on consumer health. (Šupu et al., 2015).

As for lactoferrin, it is a protein combined with iron and is found in milk and other secretions. It is found in very small quantities in breast secretions as well, such as tears, saliva, mucous fluid and genital secretions, but the largest source is milk and belongs to the iron family (Hemeprotien), and these compounds have an important and vital role in raising immunity. and antioxidants, which is sometimes called Lactotransfern (Ayah et al., 2015).

The types of lactic acid bacteria have the ability to produce some natural antibiotics towards other bacterial species, especially pathogenic ones, such as Bacteriocins, including Natamycin, which is a globally permitted food preservative. It has been used in the manufacture of cheese, meat and other food industries against molds and yeasts for many years, as it is described It is a natural mold inhibitor (Delves-Broughton, 2014).

The current study aimed to know the effectiveness of edible coatings of whey protein fortified with zinc nanoparticles (ZnNPs), natamycin and lactoferrin on some vital parameters (blood images and lipid profiles) in male laboratory rats.

Materials and methods:

Materials used:

Lactoferrin: Obtained from the American Jarrow Company, Entamycin: Obtained from the Belgian Handray Company, Nano Zinc: Obtained from the American Research Nanomaterials Company, Whey Protein: Obtained from the American Isolabs Company.

Preparation of whey protein membranes:

Whey protein membrane solutions were prepared according to the method of Carvalho and Grosso, (2004) with a weight of 10 g of whey protein powder, dissolved in 80 ml of distilled water, and mixed all components using a hot plate - Magnetic Stirrer at a temperature of 60 °C for a period of time 15 minutes, then glycerol was added at a rate of 3% of the dry weight of the whey, and the volume was added to 100 ml of distilled water and the pH was adjusted to 7.

Preparation of nanomaterials solutions with overlaps:

Concentrations of bacteriocin and lactoferrin nanomaterials were prepared from basic solutions as shown below:

Zinc nanoparticles: the concentration is 0.25 mg/100 ml prepared by adding 0.25 mg to 100 ml distilled water.

Lactoferrin: the concentration is 60 mg/100 ml prepared by adding 60 mg to 100 ml of distilled water.

Natamycin: the concentration is 50 mg/100 ml prepared by adding 50 mg to 100 ml distilled water.

Zinc nanoparticles with lactoferrin: 0.25, 60 mg/100 ml prepared by adding 0.25 + 60 mg to 100 ml distilled water.

Zinc nanoparticles with natamycin: 0.25, 50 mg/100 ml prepared by adding 0.25 + 50 mg to 100 ml distilled water.

Lactoferrin with natamycin: 60, 50 mg/100 ml prepared by adding 60+50 mg to 100 ml of distilled water.

Zinc nanoparticles with lactoferrin with natamycin: prepared by adding 0.25 + 60 + 50 mg to 100 ml of distilled water.

Bio experience:

Preparation of laboratory animals:

Healthy and disease-free laboratory animals were obtained from the College of Veterinary Medicine / University of Tikrit in numbers of 45 adult male Albino rats, 8-9 weeks old, and their weights ranged at 251-275 grams. They were distributed randomly into nine groups of similar weights, each group included five animals. The animals were placed in cages made of plastic, after spreading the floor with sawdust, which was replaced three times a week. It consists of (158.5 gm casein / kg, 100 gm glucose / kg, 50 gm cellulose / kg, 100 gm corn oil / kg, 5 gm mixture of vitamins / kg, 50 gm mixture of mineral salts / 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 dishes of stainless steel and dried in an oven at a temperature of 50 ° C by means of a hot air stream until drying is complete, then it was packed in polyethylene bags and kept in the refrigerator at a temperature of temperature (5±2) °C throughout the duration of the experiment. The animals were reared under the supervision of a specialized veterinary staff, taking into account the aspect of hygiene. They were given orally each of the ZnNPs, the compound of natamycin, lactoferrin, and the membrane only, according to the concentrations and volumes suggested in the design of the experiment.

Experiment design:

M1: control group.

M2: is the group of animals given only the rumen

M3: group animals given ZnNPs only at a concentration of 0.25 mg/100 ml.

M4: group of animals given natamycin only at a concentration of 50 mg / 100 ml..

M5: group of animals given lactoferrin only at a concentration of 60 mg / 100 ml.

M6: group of animals given ZnNPs at a concentration of 0.25 mg/100 ml with natamycin at a concentration of 50 mg/100 ml..

M7: group animals given ZnNPs at a concentration of 0.25 mg/100 ml with lactoferrin at a concentration of 60 mg/100 ml.

M8: group of animals given lactoferrin at a concentration of 60 mg/100 ml with natamycin at a concentration of 50 mg/100 ml.

M9: group animals given ZnNPs at a concentration of 0.25 mg/100 ml with lactoferrin at a concentration of 60 mg/100 ml and natamycin at a concentration of 50 mg/100 ml.

Blood tests:

After the end of the experiment, the animals were starved for 10 hours, then anesthetized using chloroform, the animals were dissected from the chest area, and blood was drawn from the heart directly to perform the necessary tests. The blood was centrifuged using a centrifuge at a speed of 3000 revolutions/min for 15 minutes to obtain serum, which was placed in 1 ml Eppendorf tubes and preserved by freezing until analyzes were performed (Tietz, 2005).

CBC blood imaging tests were performed by a French hematology analyzer. The parameters of the blood images included the number of white blood cells (WBCs), the number of red blood cells (RBCs), hemoglobin (Hb), the number of platelets (PLT), and the level of total cholesterol, triglycerides (TG) and proteins were estimated High-density lipoproteins (HDL) and low-density lipoproteins (LDL) in blood serum using a ready-made analysis kit (Kit) manufactured by the French company BlOLABO (Tietz, 2005), and analyzes were carried out by spectrophotometer (Gallenkamp) (English) and according to the recommended wavelength Analysis, and concentrations were calculated using equations according to the instructions of the company supplying each crew.

Statistical analysis:

The experiment was implemented under a complete randomized design (CRD) and analysis of variance was carried out using the general linear model within the ready-made statistical program (SAS, 2001). In the case of significant differences, Duncan's test (1955) was used to determine the significance of the differences between the different means at a probability level of 0.05.

Results and discussion:

1- Effect of oral administration of zinc nanoparticles (ZnNPs), lactoferrin, and natamycin on blood picture parameters:

Table (1) shows the effect of oral administration of ZnNPs, lactoferrin, and natamycin on blood image analysis of rats after 21 days of dosing.

Y / T	HB mg/dl	RBCs /10^6mm	PLT 10^3/mm^3
T1	0.058 ± 13.3 b	0.006 ± 5.16 b	1.155 ± 194.00 f
T2	0.058 ± 13.6 a	0.058 ± 5.21 ab	1.453 ± 202.67 e
T3	0.058 ± 13.4 c	0.035 ± 5.33 a	1.000 ± 223.83 b
T4	0.058 ± 13.4 a	0.035 ± 5.28 b	1.453 ± 211.42 d
T5	0.058 ± 12.2 e	0.018 ± 5.18 b	1.202 ± 215.53 cd
T6	0.058 ± 13.5 ab	0.035 ± 5.25 ab	0.882 ± 217.38 c

T7	0.058 ± 13.4 b	0.035 ± 5.23 ab	2.333 ± 219.45 bc
T8	0.058 ± 12.6 d	0.035 ± 5.37 b	1.732 ± 229.18 a
T9	0.285 ± 12.8 de	0.035 ± 5.30 ab	2.028 ± 223.73 b

* The numbers in the table are the average of three replicates and represent the mean values ± the standard deviation.
* The different lowercase letters within one column indicate that there are significant differences ($p \leq 0.05$) between the treatments.
T1 = control. T2 = whey protein membrane. T3 = Membrane reinforced with 0.25 mg zinc particles. T4 = membrane fortified with lactoferrin at a concentration of 60 mg. T5 = membrane fortified with 50 mg of antamycin. T6 = 0.25 membrane fortified with zinc nanoparticles at a concentration of 0.25 mg + 50 mg of natamycin. T7 = membrane fortified with zinc nanoparticles at a concentration of 0.25 mg + 60 mg lactoferrin. T=8 membrane fortified with lactoferrin at a concentration of 60 mg + 50 mg of neptomycin. T9 = Membrane fortified with zinc nanoparticles at a concentration of 0.25 mg + 60 mg lactoferrin + 50 mg natamycin.

Table (1) shows the effect of oral dose of zinc nanoparticles ZnNPs, lactoferrin, and entamycin on blood picture parameters of laboratory rats for a period of 21 days, The results showed that hemoglobin levels in the blood of rats for treatments T3, T4, T5, T6, T7, T8, T9 were 13.4, 13.2, 12.2, 13.5, 13.4, 12.6, 12.8 mg/dl, respectively and differed significantly ($p<0.05$) compared to With control samples T1 and T2 which were at 13.3 and 13.6 mg/dl respectively. Amr et al. (2016) confirmed that exposure of laboratory animals to zinc nanoparticles for a period of 7-28 days leads to an increase in hemoglobin concentration, as well as the volume of red blood cells and the number of red blood cells. It also agreed with what Zarzour (2014) mentioned that the hemoglobin concentration ranged between 12.9-13.0 mg/dl when adding gelatin, natmycin, EDTA, and sorbic. As well as with what Rosa et al. (2017) indicated that lactoferrin, when used in pregnant women, raises and improves blood picture parameters.

The results showed that the effect of oral administration of zinc particles ZnNPs, lactoferrin and nitmycin on the number of red blood cells (RBCs) showed a significant difference ($p < 0.05$) compared with its values in the control group, which was 5.19 × 106 / mm, zinc is present in the blood at a ratio between -70 125 mg/100 milliliters (Al-Sharbaji, 2015).

Table (1) showed that the number of platelets in the blood of rats given ZnNPs, lactoferrin, and natmycin for treatments T3, T4, T5, T6, T7, T8, T9 were at 223.83, 211.42, 215.53, 217.38, 219.45, 229.18, 223.73 109/L, respectively, compared With their numbers in control group T1 and T2 which were 194 and 202.67 103/mm3 respectively. These results agreed with what was mentioned by Espinosa et al. (2013) that the increase in platelet count was 42.33 103/mm3. These results converged with Al-Jubouri (2022) that the blood platelets in laboratory rats dosed with nanoparticle zinc and the probiotic were between 320-373.103/mm3 The results agreed with what was mentioned by Espanani et al., (2015) who showed that the addition of nanoparticle zinc, It showed a significant increase in the concentration of hemoglobin and the number of red blood cells. These results also agreed with what was found by Amr et al., (2016) that exposure of laboratory animals to zinc nanoparticles for a period between 7-28 days led to an increase in hemoglobin concentration, the volume of packed red blood cells, and the total number of red blood cells.

Hassan et al. (2022) stated that lactoferrin works to support and enhance the size of cells, adjust the proportion of red blood cells within normal limits, and reduce the level of fats in the blood, The reason for the increase in the number of red blood cells and the concentration of hemoglobin and platelets when lactoferrin is added may be due to its role in increasing the efficiency of the immune system, enhancing the absorption of mineral elements and activating the process of producing amino acids.

The reason for the increase in the total number of red blood cells and the concentration of hemoglobin when adding zinc nanoparticles may be due to many reasons, including that it has the ability to reduce the

toxicity of compounds, as well as the fact that it acts as an antioxidant and improves blood, chemical and biological changes (Adamcakova-Dodd et al., 2014). The improvement in the measured values may be attributed to the protective effect, antioxidant nature, and immunomodulation of neptomycin, which plays a significant role in maintaining homeostasis and lowering blood pressure, Netmycin works indirectly by stimulating the bone marrow cells towards the formation of red blood cells, which helps in treating anemia and enhancing the general health of the body.

The reason for the increase in the percentage of packed cells and hemoglobin concentration can be attributed to the role of antioxidants in stimulating red blood cell production centers as they are regulators of some iron molecules by forming protoporphyin and is a good receptor for iron that enters directly into the manufacture of blood and thus leads to an increase in the number of Red blood cells in the body, in turn, lead to a positive increase in hemoglobin concentration.

2- Effect of oral administration of ZnNPs, natamycin, and lactoferrin to male laboratory rats on white blood cells of rats:

Figure (1) shows the effect of oral administration of ZnNPs, natmycin and lactoferrin on white blood cells of male laboratory rats for 21 days alone or together. The results showed that there was a significant decrease ($P<0.05$) in the values of white blood cell numbers, where T2, T3, T4, T5, T6, T7, T8, T9 were between (7.18-9.70) mm3/106, respectivelym Compared with its numbers in the control sample, which was at 10.48 mm3/106. These results coincided with what Eman et al. (2021) found that the effect of zinc nanoparticles given during the dosing process to laboratory animals reduced the level of white blood cells to normal levels. Ahmed et al. (2021) indicated that lactoferrin works to increase the blood rate and eliminate anemia.

White blood cells are important as they are considered one of the body's immune systems and have a vital role in protecting the body from diseases caused by viral, parasitic and bacterial infections, as their rise is an indication of infection, the presence of foreign bodies or antigens in the circulatory system, disorders in the immune system, or an infection that occurred in the body (Yunus, 2017).

The results of the current study were contrary to the study of Tang et al. (2016) in which feeding rats with ZnO nanoparticles at the levels of 300 or 600 mg/kg significantly increased the levels of alkaline phosphatase and beta-glutamyltransferase, which they suggested was due to liver injury produced by the nanoparticles, This is supported by histopathological evidence of liver injury, which was observed in the group fed with 300 mg/kg of nanoparticles and increased in severity in the group fed with 600 mg/kg of nanoparticles, As they found an increase in the number of white blood cells, neutrophils, and granulocytes, which reflects an inflammatory reaction, it also agreed with what was found by Khan et al. (2013) that dosing rats with probiotics at different concentrations led to a decrease in the total number of white blood cells and an increase in lymphocytes.

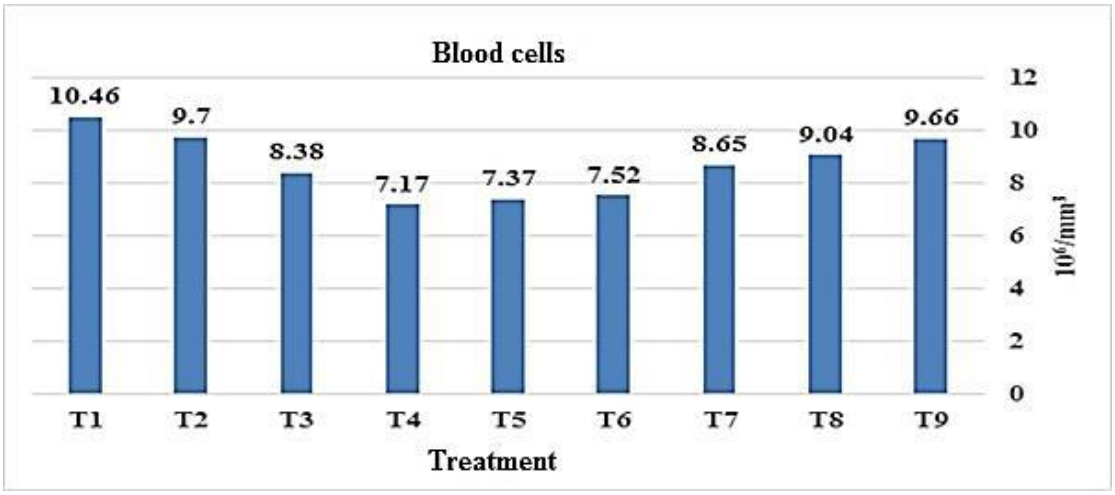

Figure (1): Effect of oral administration of ZnNPs, neptomycin, and lactoferrin to male laboratory rats on white blood cells.

3- Effect of oral administration of ZnNPs, lactoferrin and natamycin on lipid profile:

Table (2) shows the effect of the interaction between ZnNPs, lactoferrin, and natamycin for male rats given orally for 21 days on the lipid profile. The results showed that there was an increase in the values of cholesterol and triglycerides ($p<0.05$) in the blood of male laboratory rats given orally, compared to the control samples T1 and T2, where they were 80.80 and 84.33 mg/dl, respectively. While the concentration in the rest of the treatments ranged between 94.67-103.40 mg/dL.

As well as with the triglycerides TG, where the results showed a significant decrease ($p<0.05$) for the treatments T3, T4, T5, T6, T7, T8, T9, as they reached 69.43, 63.95, 61.72, 65.64, 65.76, 64.39, 64.31, 62.89 mg/dL respectively, compared with control group T1 and T2 where they were 79.43 and 80.27 mg/dL, respectively.

The results showed that the concentrations of high-density lipoprotein (HDL) had a significant increase in all groups of rats given orally for a period of 21 days, Where the values of the treatments T2, T3, T4, T5, .T6, .T7, T8, T9 were at 35.43, 41.93, 43.52, 44.64, 43.73, 44.25, 44.32 and 45.12 mg/dl, respectively, compared with the control treatment T1, which was 33.80 mg/dl.

The results showed that the LDL values had a significant decrease in all treatments compared with the control samples T1 and T2, which were at 26.47 and 27.12 mg/dl, respectively.

The results showed a significant increase ($p<0.05$) in the low-density lipoprotein (VLDL) compared with the control samples T1 and T2, where it was 15.34 and 16.63 mg/dl, respectively. The results agreed with what Zheng et al. (2018) found that feeding with whey protein-fortified casings led to an increase in high-density lipoprotein cholesterol and a decrease in low-density lipoprotein cholesterol, as well as a decrease in fat-producing enzymes in the liver, and this leads to a reduction in atherosclerosis. The results agreed with what Morishita et al. (2013) found that the use of lactoferrin reduced triglyceride levels in the liver as well as in the body of rats. Lactoferrin regulates metabolic balance, reduces cholesterol synthesis, and improves metabolism (Min et al., 2018).

It also agrees with Hassan et al. (2022) who found that a lactoferrin-fortified diet was beneficial in weight loss and in improving lipid profile by restoring cellular antioxidants, resulting in fat loss. Jusni et al. (2022) found that feeding rats with doses of 100, 200, and 400 mg/kg of body weight of lactoferrin can reduce total cholesterol and triglyceride levels to normal levels, and showed that lactoferrin can significantly reduce cholesterol levels in the liver of rats. Lactoferrin enhances fat absorption by stimulating bile acid synthesis and inhibiting the lactoferrin pathway to produce TNF-α (Superti, 2020). Lactoferrin also acts as a catalyst for the AMPK protein, which regulates energy balance and coordinates metabolic pathways, thus balancing nutrients. This protein has a role in converting cholesterol into bile acids via protein ketase activated by the enzyme CyP7A1, which in turn contributes to improving liver and kidney function (Chiang and Ferrel, 2020).

Fat metabolism is a complex process involving several enzymes such as Fatty Acid Synthase (FAS) and Acetyl-CoA Carboxylase (ACC), which are the main enzymes for fatty acid synthesis, and Adipose triglyceride lipase (ATGL) which is the main enzyme for triglyceride degradation and all of these are controlled Enzymes by AMP-activated protein kinase (AMPK) (Wang et al., 2015), as Min et al. (2018) found that taking lactoferrin inhibits the action of FAS and ACC and activates the enzyme action of ATGL, thus reducing lipogenesis and increasing lipolysis in the liver and thus to improve Lipid metabolism: Lactoferrin and metformin when fed to rats decreased TG levels in the liver, indicating that they may work synergistically to improve lipid metabolism.

The results agreed with what Ahmed (2020) found that giving laboratory rats with hyperlipidemia zinc nanoparticles and nisin led to a significant decrease in the concentration of TG, LDL, VLDL, and an increase in the concentration of HDL in the treatments.

The results of this study agree with what was mentioned by Mahmoud et al. (2021) that treatment with ZnONPs reduced body weight by inhibiting the increase in total cholesterol and triglycerides and increasing the level of HDL, This antilipidemia effect may be due to regulating cholesterol metabolism in the liver and reducing oxidative stress. These results also agree with what was mentioned by Stawarska et al. (2021) that feeding with zinc, zinc and nanoparticles led to a significant decrease in cholesterol content in the serum of rats compared to the control group.

The study of Shkal et al. (2020) showed that rats fed with ZnO NPs recorded a significant decrease

in total cholesterol, triglycerides, and LDL, and a significant increase in HDL in the blood. These results indicate that zinc plays a major role in regulating the action of a group of enzymes involved in the digestion and absorption of fats (Gadoa et al., 2022).

Table (2-2): shows the effect of oral administration of zinc nanoparticles ZnNPs, lactoferrin and natamycin to male laboratory rats for a period of 21 days on the parameters of lipid profiles.

Y / T	VLDL (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	TG (mg/dl)	Chol (mg/dl)
T1	0.006 ± 15.34 e	0.009 ± 26.47 b	0.058 ± 35.43 i	0.058 ± 79.40 b	0.058 ± 90.80 i
T2	0.060 ± 16.63 d	0.012 ± 27.12 a	0.058 ± 41.96 h	0.058 ± 80.20 a	0.058 ± 94.33 h
T3	0.038 ± 11.07 h	0.006 ± 18.68 h	0.058 ± 43.51 g	0.058 ± 69.43 c	0.384 ± 83.87 e
T4	0.032 ± 14.88 f	0.006 ± 20.11 g	0.058 ± 44.62 f	0.058 ± 63.95 f	0.058 ± 85.63 g
T5	0.032 ± 15.39 e	0.006 ± 22.16 c	0.058 ± 44.62 c	0.058 ± 61.72 h	0.115 ± 82.76 a
T6	0.035 ± 15.06 b	0.006 ± 21.72 f	0.058 ± 43.73 e	0.058 ± 65.83 d	0.285 ± 87.66 f
T7	0.026 ± 15.56 a	0.006 ± 21.86 d	0.058 ± 44.26 d	0.058 ± 64.38 e	0.058 ± 88.48 b
T8	0.026 ± 15.20 g	0.006 ± 21.92 e	0.058 ± 44.31 b	0.058 ± 64.29 g	0.058 ± 82.16 d
T9	0.017 ± 12.93 c	0.009 ± 21.77 e	0.058 ± 45.12 a	0.058 ± 62.88 f	0.058 ± 83.45 c

* The numbers in the table are the average of three replicates and represent the mean values ± the standard deviation.
* The different lowercase letters within one column indicate that there are significant differences ($p \leq 0.05$) between the treatments.
T1 = control. T2 = whey protein membrane. T3 = Membrane reinforced with 0.25 mg zinc particles. T4 = membrane fortified with lactoferrin at a concentration of 60 mg. T5 = membrane fortified with 50 mg of antamycin. T6 = 0.25 membrane fortified with zinc nanoparticles at a concentration of 0.25 mg + 50 mg of natamycin. T7 = membrane fortified with zinc nanoparticles at a concentration of 0.25 mg + 60 mg lactoferrin. T=8 membrane fortified with lactoferrin at a concentration of 60 mg + 50 mg of neptomycin. T9 = Membrane fortified with zinc nanoparticles at a concentration of 0.25 mg + 60 mg lactoferrin + 50 mg natamycin.

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