

Effect of Coating Hatching Eggs with Nanoparticles and Carboxymethyl Cellulose and Storage periods on Hatchability and Quality of Hatched Chicks

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Abstract

The current research aimed to conducting two experiments to study the effect of coating hatching eggs with nano-titanium dioxide (nano-TiO₂) and nano-silica dioxide (nano-SiO₂) particles and their mixture with carboxymethyl cellulose (CMC) on the characteristics of hatching percentage, embryo growth inside the egg. The study was conducted in the Department of Animal Production, College of Agriculture, Tikrit University for the period from 19/3/2023 to 17/9/2024. It aimed to evaluate the coating of hatching eggs with Nano-TiO₂ and Nano-SiO₂ particles and their mixture with carboxymethyl cellulose CMC on the qualities of hatching percentage, embryo growth inside the egg, as well as trying to obtain the best and longest storage method for fertilized eggs up to 10 days and evaluating embryo growth and gene expression of growth genes during the egg incubation period. The results revealed that the process of coating unstored hatching eggs with different coats led to a significant improvement ($P<0.05$) in the hatching percentage from 90% for control treatment eggs to more than 91% for coated eggs, in addition to a decrease in early, medium and late mortality, and compressed eggs, and an increase in hatched chicks' parameters, as the average chick weight increased from 43.83 g in control treatment eggs to more than 47 g for eggs coated with nano-coating treatments, and all hatched chicks' parameters improved. The process of coating hatching eggs with different coats and storing them in the refrigerator for 10 days led to a significant improvement ($P<0.05$) in the hatching percentage from 86.33% for control treatment eggs to more than 91% for coated eggs, in addition to a decrease in early, medium and late mortality, compressed eggs, and an increase in hatched chicks' parameters, as the average chick weight increased from 42.15 g in control treatment eggs to more than from 47 grams of eggs, the egg coating treatments with nano-coatings improve all indicators of hatched chicks.

Keywords: Hatching Eggs, Coating, Nanoparticles, CMC, Hatching percentage, Chick's quality.

This research is a part of PhD thesis of the first author.

Introduction

The egg is the reproductive cell in birds and some reptiles and is also a staple food for humans (1). Although it's hard, calcareous shell plays an essential protective role, it can deteriorate in quality or be penetrated by microorganisms, threatening the safety of both table and hatching eggs (2, 3). With the rapid development of the poultry industry and the increasing global demand for its products, maintaining egg quality during transportation and storage has become a major challenge, especially in developing countries that lack adequate preservation methods (4). Storage at room temperature leads to a decline in internal egg quality (5), while the United States Department of Agriculture recommends immediate refrigeration after collection (6). Recently, studies have focused on the use of nanomaterials as a promising method for egg preservation, due to their large surface area, high catalytic efficiency, and strong adsorption capacity (7). Applications of nanoparticles such as TiO_2 and SiO_2 have shown positive results in extending storage life, reducing microbial contamination, enhancing nutritional value, improving embryo and chick viability, and maintaining the hardness and quality of the calcareous shell (8, 9).

Despite the importance of hatching eggs in the poultry industry, rapid deterioration in their quality during storage and exposure to microbial Hatching egg packaging experiments on hatching traits:

First experiment: The eggs from the first experiment were brought and treated on the same day, with 900 eggs, 150 eggs per treatment. Incubation was carried out on the same day, September 30, 2023.

The second experiment: Eggs were brought from the same source from mothers at the age of 35 weeks, with a total of 900 eggs, 150 eggs for each treatment as well. They were treated and

contamination are among the most significant obstacles limiting their hatching and embryo development efficiency.

This work aims to study the effect of spraying hatching eggs with nanoparticles (TiO_2 and SiO_2) and mixing them with carboxymethyl cellulose (CMC) in improving hatching rates, increasing the efficiency of embryo growth inside the egg, and reducing problems associated with shell quality and microbial contaminants.

Materials and Methods

The study was conducted at the Department of Animal Production, College of Agriculture, Tikrit University, from March 19, 2023, to September 17, 2024. The study aimed to evaluate the effects of coating table and hatching eggs with nano- TiO_2 and nano- SiO_2 particles, and their mixture with carboxymethyl cellulose (CMC), on hatchability and embryo growth within the egg. The study also sought to develop the best and longest storage method for fertilized eggs, up to 10 days, and to evaluate embryo growth during the incubation period.

Hatching Eggs: One-day-old eggs were obtained from the farm of Vano Company, operating in Erbil Governorate, Kurdistan Region, and northern Iraq, which specializes in raising broiler (Ross 308) egg-producing mothers. The eggs were obtained and the experiments were conducted over two periods.

stored on 6/12/2024 AD, for ten days at a temperature of ($1^\circ\pm 13^\circ$) and humidity of (70-75)%. After that, the eggs were incubated on 6/22/2024 AD. Regarding the average weight of the eggs, it ranged between (67-70) grams for both experiments.

Egg Incubation: For storage eggs, they were removed from the refrigerator after completing the 10-day storage period and were warmed before being placed in the incubator. The eggs,

along with the treated day-old eggs for the second experiment, were placed in the incubator with the wide end facing up in a Petersim incubator with a capacity of 4200 eggs per batch in trays of 150 eggs each. The temperature was set at 37.7°C and the humidity was 55-60% for the first 18 days, with continuous turning at two 45-degree angles, as stated in the incubator manufacturer's manual.

Hatchery: A single-stage Petersim incubator with a capacity of 4200 eggs was also used, in the Animal Production Department Field Hatchery / College of Agriculture / Tikrit University. Cleaning and sterilization operations were carried out for the hatchery and the incubator with all its parts before and after the hatching process. A continuous and controlled electrical source was provided to ensure that the electrical current would not be cut off, in addition to

continuous monitoring. The temperature was programmed at 37.7°C and the humidity (75-80) % to obtain the best hatching percentage.

Experimental Treatments

Chemicals used in packaging

Carboxymethylcellulose: (CMC) is a water-soluble, anionic polysaccharide derived from cellulose that is used as a thickener and stabilizer in various applications. Also, it is generally considered safe and has a long history of use. Food-grade carboxymethyl cellulose was obtained from the local market in Baghdad.

Nanocomposites: Both nano-titanium dioxide (nano-TiO₂) and nano-silica dioxide (nano-SiO₂) were nanoparticles with distinct properties and applications. These obtained in collaboration with Northeast Agricultural University (NEAU) in China (Figure 1).



Figure (1): Image of Nano-TiO₂ and Nano-TiO₂ composite powder nano-titanium dioxide (nano-TiO₂), nano-silica dioxide (nano-SiO₂)

The first treatment, T1, was the control treatment in which nothing was used to coat the eggs.

The second treatment, T2, was prepared with 0.5% carboxymethyl cellulose (CMC) solution. A 500-ml glass beaker containing a ready-made normal saline solution was prepared, and (2.5 g)

of CMC was gradually added to it to prevent clumping. The mixture was stirred continuously with a magnetic stirrer at a temperature of 25°C for a quarter of an hour until it was completely dissolved and became a clear solution (9, 10).

The third treatment T3: Prepare the solution by gradually dissolving (1g) of Nano-SiO₂ into

(500) milliliters of ready-made medical Normal Salin solution in a glass beaker with continuous stirring using a magnetic stirrer for a quarter of an hour to obtain a concentration of 0.2% (9).

The fourth treatment T4: By mixing 500 ml of a carboxylic acid solution such as cellulose (CMC) at a concentration of 0.5% as in the first paragraph, then we add (1g) of Nano-SiO₂ to it, and mix the mixture for a quarter of an hour on a magnetic stirrer to obtain a concentration of 0.2%.

The fifth treatment T5: The solution was prepared by gradually dissolving (1 gram) of Nano-TiO₂ into (500) ml of ready-made medical Normal Saline solution in a glass beaker with continuous stirring using a magnetic stirrer for a quarter of an hour to obtain a concentration of 0.2% (9).

The sixth treatment T6: To obtain a solution with a concentration of 0.2% of Nano-TiO₂ and a concentration of 0.5% of CMC, then we dissolve (1) gram of Nano-TiO₂ in 500 ml of a solution of carboxylic acid such as cellulose (CMC) with a concentration of 0.5% in a beaker on a magnetic stirrer for a quarter of an hour to obtain a solution with a concentration of 0.2% of Nano-TiO₂ and a concentration of 0.5% of CMC.

Hatching traits studied:

Hatchability percentage from fertilized eggs:

Calculated according to the following equation:
chicks

$$\text{Hatchability percentage} = \frac{\text{Number of hatched}}{\text{Number of fertilized eggs}} \times 100$$

Embryonic mortality percentage: Embryonic mortality was calculated based on the Dutch poultry hatchery company (Petersim) manual as follows:

Early embryonic mortality: (1-11 days), embryos lacking down.

Intermediate embryonic mortality: (12-18 days), embryos containing down.

Late embryonic mortality: (19-21 days), embryos fully developed but unable to hatch.

Percentage of Pecking Embryos:

Live Pecker: Includes chicks that have pecking and cracked the shell but are unable to emerge.

Dead Pecker: Includes chicks that have fully developed and pecking the eggshell but are unable to crack it.

Chick Weight: The chick's weight is measured individually using a scale and sensor.

Tona Scale: Chicks were graded according to the criteria adopted by Tona 2005 (11). These included:

Activity (0-6) and are assessed by turning the chicks onto their backs and observing their speed of returning to their normal position.

Fluff condition (0, 8, 10) and are assessed by measuring the amount of fluff, including light, missing, and dirty (contaminated) fluff.

Eye condition (0, 8, and 16) and are assessed by observing sunken eyes.

Legging condition (0, 16) and are assessed by examining the legs and the presence or absence of a red spot on the foot.

Navel condition (0, 6, 12) and are assessed by turning the chick onto its back and carefully observing the navel to ensure its condition.

Remaining membranes (0, 4, 8, and 12) and are assessed using the same method as the navel.

Yolk decay: (0, 12) degrees. This is assessed by having the chick stand upright on the hand, then placing the left thumb under the neck and pushing it forward with the end of the left hand to present the chick's abdomen. Examine the abdomen to determine whether the yolk is decayed.

Residual yolk: (0, 8, 12, 16) degrees. This is assessed using the same method as the examination above.

Body mass: Body mass was calculated as described by Willemsen et al., 2008 (12), using the equation: = chick weight / chick length.

Chick length from beak to third toe: This measure was measured using a ruler graduated from 1 to 30 cm. The chick was turned onto its belly and measured from the nail on the third toe to the beak.

Chick length from head to tail (CRL): Measured similarly to the previous measure, but from the head to the tail.

Leg length: Measured from the leg joint to the beginning of the nail (Leg+Toe).

Toe length: The length of the middle toe was measured using a ruler graduated from 1 to 30 cm.

Statistical analysis: All data were analyzed using the SAS 2001(13) statistical program, where the ANOVA table one-way statistical analysis was applied, and the results were compared using Duncan Multiple Range Test 1955 (14).

Results and Discussion

Hatching characteristics of packaged eggs without storage: Table (1) shows the effect of table egg packaging treatments on hatching characteristics, early, intermediate, and late mortality rates, and the percentage of broiler chicks hatched from broiler mothers without storage. The hatching rate from fertilized eggs reached 90% for the first treatment, the control treatment, with a significant difference from the rest of the egg packaging treatments, which in turn significantly outperformed the control treatment, with values ranging between 90.67 and 91.67%. The early, intermediate, and late mortality rates, as well as the percentage of broiler chicks, were significantly higher in the control treatment, followed by the second treatment, with the nanomaterial coating treatments recording lower rates, with a significant difference from the rest of the treatments.

Table (1) The effect of egg packaging treatments compared to the control treatment on hatching characteristics of hatched chicks and embryonic deaths from incubated hatching eggs from ROSS 308 broiler mothers (without storage)

Treatments	Hatching from fertilized eggs %	Early mortality%	Intermediate mortality%	Late mortality%	Pipped chicks%
T1	0.01 ± 90.00 b	± 1.00 0.01 a	1.33 ± 0.01 a	2.33 0.01 ± b	0.01 ± 6.33 a
T2	0.01 ± 91.00 a	0.00 ± 0.33 b	0.66 ± 0.01 b	1.67 ± 0.01 c	5.67 ± 0.00 b
T3	0.02 ± 91.00 a	0.67 ± 0.01 ab	0.33 ± 0.00 c	2.00 ± 0.01 b	5.67 ± 0.01 b
T4	0.01 ± 90.67 ab	0.33 ± 0.00 b	0.00 ± 0.00 c	2.00 ± 0.01 b	6.00 ± 0.00 ab
T5	0.03 ± 91.67 a	0.00 ± 0.00 c	.33 ± 0.02 c	.67 ± 10.03 c	5.67 ± 0.03 b
T6	0.01 ± 91.67 a	0.67 ± 0.01 a	0.53 ± 0.00 c	2.33 ± 0.01 b	5.00 ± 0.01 b
Average qualities	0.01 ± 91.40	0.00 ± 0.66	0.00 ± 0.72	0.00 ± 2.27	0.00 ± 5.33

Different lowercase letters indicate significant differences between treatments. * $P < 0.05$.

Hatched chicks characteristics (without storage): Table (2) shows the effect of table egg packaging treatments on the rates of hatched chicks characteristics, which included chick weight, relative weight, chick length from head to toe, chick length from head to tail, leg length from joint to toe, finger length, and body mass (Tuna specifications) according to the Tuna scale from broiler mothers' hatching eggs, where it is clear that the different egg packaging treatments significantly outperformed ($P<0.05$) the control treatment, which is the first treatment that recorded the lowest chick weight after hatching, reaching 43.83 g, while the second, third, fourth, and fifth treatments recorded higher values, reaching 46.88, 45.58, 47.34, 45.73, and 47.34 g, respectively, with a significant difference from the control treatment. Egg packaging treatments also outperformed the relative chick weight and also in the rest of Tuna characteristics, and the body mass was Tuna's egg specifications were superior in all egg packaging treatments compared to the control. Prolonged egg storage negatively impacts hatchability, as prolonged storage reduces embryo vitality and increases the mortality rate within the egg, leading to a decrease in the number of hatched chicks. In addition, improper storage may affect the

quality of the resulting chicks, which may be weaker or more susceptible to disease. Hatching fresh chicken eggs without storage increases hatchability by increasing embryo vitality and decreasing mortality rates. It also improves the quality of the hatched chicks, which are heavier, livelier, and less susceptible to disease. Therefore, eggs intended for hatching should be collected regularly, preferably several times a day, especially on hot days (15, 16).

The Tuna traits of hatched chicks, which specifically refer to the work of the researcher (11), are a set of qualitative and quantitative characteristics used to evaluate the quality of one-day-old chicks. These traits are used to determine the general health and viability of chicks and can be affected by various factors such as incubation conditions and the age of the breeder flock. We note that these traits, listed in Table (2), were superior in egg coating treatments, especially those coated with nanocomposites. This is because egg coating reduces moisture loss from the egg during hatching, thus preventing the embryos from drying out and adhering to the eggshell, increasing the hatchability and quality of hatched chicks. Nanomaterials may also increase the activity of internal enzymes in hatched embryos.

Table (2) The effect of egg packaging treatments compared to the control treatment on the characteristics of chicks hatched from incubated eggs from ROSS 308 broiler (without storage)

Treatments	Tona scale							
	Chick weight g	Relative chick weight%	Length of chick from head to toe / cm	Length of chick from head to tail CRL/cm	Leg length from joint to toe/cm	Finger length cm	body mass	Specifications Tona
T1	43.83± 0.07 c	0.68 ±0.01 b	0.02 ± 18.80 b	0.33 ±12.33 b	0.33 ± 2.23 a	0.06 ± 5.100 b	0.09 ± 2.33 d	0.57 ± 97.01 b
T2	46.88 ± 0.49 a	0.01 ± 0.73 a	19.420.44 ± a	± 13.130.13 a	2.23 ± 0.03 a	5.23 ± 0.12 a	2.54 ± 0.05 b	99.97 ± 0.71 a
T3	45.58 ± 0.22 b	0.71 ± 0.00 ab	19.17 ± 0.23 a	13.83 ± 0.33 a	2.20 ± 0.06 a	5.43 ± 0.13 a	2.52 ± 0.04 b	100.00 ±0.76 a
T4	47.34± 0.19 a	0.73 ± 0.01 a	19.17 ± 0.17 ab	13.83 ± 0.07 a	2.27 ± 0.03 a	5.23 ± 0.17 a	2.61 ± 0.07 a	100.00 ± 0.41 a
T5	45.73 ± 0.37 b	0.73 ± 0.02 a	19.17 ± 0.03 a	13.00 ± 0.33 a	2.20 ± 0.00 a	5.43 ± 0.12 a	2.52 ± 0.06 b	100.00 ± 0.35 a
T6	47.34 ± 0.15 a	0.71 ± 0.00 a	19.17 ± 0.17 a	12.93 ±0.07 a	2.33 ± 0.09 a	5.03 ± 0.03 ab	2.44 ± 0.03 c	99.97 ± 0.33 c

Different lowercase letters indicate significant differences between treatments. * $P < 0.05$.

Hatching characteristics of coated eggs (10-day storage): Table (3) shows the effect of hatching egg packaging treatments on the hatching rate of fertilized eggs and the rates of early, medium and late embryonic deaths and capsulated eggs for broiler mothers' hatching eggs after storage for 10 days. The first treatment, which is the control treatment, recorded the lowest hatching rate, reaching 86.33%, followed by egg packaging treatments, which recorded values ranging between 90.00 - 91.33%, with a significant difference ($P<0.05$) from the first treatment. The early mortality rate was also significantly high in the eggs of the first treatment, while it was low for the eggs of the other five egg packaging treatments. The medium mortality, late mortality and capsulated chicks recorded the highest rates in the control treatment, reaching 0.67, 4.00 and 7.00%, respectively. The egg packaging treatments contributed to reducing the rates of these deaths and increasing Hatching rate: The nano-egg coating treatments recorded better values compared to the second and first treatments and the differences between them were not significant. From the table, we note that the best hatching characteristics were recorded by the

sixth treatment, which coated the hatching eggs using carboxymethyl cellulose + nano-titanium dioxide, with no difference from treatments four and five. Regarding the hatching rate of fertilized eggs, treatment six achieved the highest expected hatching rate, thanks to providing maximum protection against infection and good control of moisture loss (despite ventilation challenges). The control treatment, on the other hand, represented the normal level without intervention. The hatching rate was good, but it was susceptible to decline due to contamination or suboptimal conditions. Regarding early mortality, the coatings of treatments four and six played an effective role, preventing leakage. Table (4) shows the effect of hatching egg packaging treatments on the rates of hatched chick characteristics according to the Tuna scale from hatching eggs of broiler mothers stored for 10 days. We find that the first treatment, which is the control treatment, recorded the lowest Tuna scale values, as the chick's weight reached 42.15 g, with a significant difference ($P<0.05$) from the rest of the egg packaging treatments, which recorded values ranging between 44.05 - 47.22 g, respectively.

Table (3) The effect of treatments compared to the control treatment on hatching characteristics and embryonic mortality of chicks hatched from eggs hatched from ROSS 308 broiler (storage for 10 days)

Treatments	Hatching from fertilized eggs %	Early hatching %	Intermediate hatching %	Late hatching %	Pipped chicks%
T1	0.01 ± 86.33 b	±2.67 0.01 a	±0.670.00 a	4.00 ± 0.00 a	0.01 ± 6.33 a
T2	0.01 ± 90.00 a	0.01 ± 1.67 b	0.000. ±01 c	± 1.000.00 c	5.67 ± 0.01 b
T3	0.01 ± 90.33 a	1.67 ± 0.01 a	0.00 ± 0.00 c	1.00 ± 0.01 c	4.33 ± 0.01 c
T4	0.01 ± 90.33 a	1.33 ± 0.01 a	0.00 ± 0.00 c	0.67 ± 0.00 c	5.00 ± 0.01 b
T5	0.01 ± 90.33 a	1.67 ± 0.01 b	0.00 ± 0.01 c	2.00 ± 0.01 b	5.33 ± 0.00 b
T6	0.01 ± 91.33 a	1.33 ± 0.01 a	0.33 ± 0.00 b	1.00 ± 0.01 c	4.33 ± 0.01 c
Average qualities	0.01 ± 89.78	0.01 ± 1.72	0.00 ± 0.17	0.00 ± 1.61	0.01 ± 5.28

Different lowercase letters indicate significant differences between treatments. * $P < 0.05$.

The relative chick weight was also low in the first treatment compared to the other five egg packaging treatments. The same table also indicated that the traits of chick length from head to toe, chick length from head to tail, leg length from knuckle to toe, toe length and body mass were very low in hatching eggs stored for 10 days in the control treatment, with

values ranging from 0.63, 19.35, 12.28, 1.86, 4.96, 2.18 and 94% for the mentioned traits, respectively. Meanwhile, the egg packaging treatments recorded higher values for tuna traits, especially the third, fourth, fifth and sixth treatments, as the general tuna specifications indicated that they achieved 100%.

Table (4) The effect of treatments compared to the control treatment on the characteristics of hatched chicks according to the Tuna scale from ROSS 308 broiler hatching eggs (storage 10 days)

Treatments	Tona scale							
	Chick weight g	Relative chick weight %	Length of chick from head to toe / cm	Length of chick from head to tail CRL/cm	Leg length from joint to toe/cm	Finger length cm	Body mass	Specifications Tona
T1	42.15± 0.68 c	0.01± 0.63 c	19.35± 0.18 b	12.28± 0.18 b	1.86± 0.06 c	4.96± 0.05 c	2.18± 0.03 b	94± 0.68 b
T2	47.220.79 ± a	0.01 ± 0.72 a	19.81± 0.12 a	12.91± 0.68 a	1.88± 0.03 bc	5.01± 0.04 bc	2.39± 0.01 a	99± 0.48 a
T3	44.05 ± 0.54 b	0.66 ± 0.01 bc	19.77± 0.09 ab	12.78± 0.08 a	2.07± 0.08 a	5.17± 0.07 ab	2.23± 0.02 b	100± 0.00 a
T4	46.08 ± 0.31 a	0.70 ± 0.01 ab	19.58± 0.06 ab	13.06± 0.17 a	2.03± 0.08 ab	5.20± 0.05 a	2.36± 0.02 a	100± 0.00 a
T5	47.06 ± 0.78 a	0.69 ± 0.02 ab	19.82± 0.09 a	12.92± 0.04 a	1.95± 0.02 ab	5.28± 0.08 a	2.37± 0.03 a	100± 0.00 a
T6	45.66 ± 0.05 ab	0.70 ± 0.01 ab	19.60± 0.15 ab	12.96± 0.06 a	1.97± 0.02 ab	5.22± 0.03 a	2.32± 0.08 a	100± 0.00 a
Average qualities	45.020.02 ±	49.06 ± 0.72	49.06 ± 19.08	49.06 ± 12.86	49.06 ± 2.23	49.06 ± 5.07	49.06 ± 2.36	49.06 ± 92.00

Different lowercase letters indicate significant differences between treatments. * $P < 0.05$.

Fertilized eggs are a delicate biological system that requires a tight balance between gas exchange (oxygen and carbon dioxide), moisture loss, and protection from microbial infection to ensure embryo development and successful chick hatching. The structural composition of the eggshell (calcite layers, pores, and the natural cuticle) directly influences these vital processes. During incubation, the embryo's oxygen requirements gradually increase until they peak as hatching approaches. A certain percentage of the egg's water is also required to create a sufficient air volume within the egg to prevent the embryo from suffocating or drowning upon hatching. At the same time, the shell and cuticle form a barrier against bacteria, preventing most microbes from penetrating the egg, while allowing some gas exchange and moisture necessary for embryo survival. Any modification to the shell surface—such as coating eggs with different materials—will necessarily affect the rates of gas exchange, water loss, and microbial ingress, which may be reflected in hatchability and embryo loss rates at different stages of incubation (17).

In summary, the above results show that each treatment had a distinct effect on the embryo's in-egg environment, which was reflected in hatching outcomes. Treatments containing CMC membranes provided the greatest protection against infection but suffered from problems providing sufficient oxygen to the embryos during late incubation. The addition of nanoparticles to the CMC improved the results relatively by increasing microbial protection, particularly TiO_2 , and possibly modifying the membrane's permeability.

Treatments containing only particles (without a polymer) had less pronounced overall effects; they did not severely suffocate the embryos, but they did not fully protect them either. The best balance was achieved in treatment 6, which nearly eliminated early losses and increased the number of embryos that reached hatch, partially offsetting the increased late losses resulting from excessive isolation (9).

The duration of storage of chicken eggs significantly affects the hatchability and vitality of chicks. The longer the storage period, the lower the hatchability, the higher the embryo mortality rate, and the weaker the vitality of hatched chicks. The effect of storage depends on the storage period, as storage for up to 7 days does not usually significantly affect the hatchability if stored at an appropriate temperature and humidity (15°C and 75% humidity) (18). While increasing the storage period to 7-14 days, the hatchability rate begins to decrease significantly, and the percentage of embryos that die during this period may increase. It has been observed that increasing the storage period of eggs to more than 20 days can result in the death of most embryos, and the eggs may not hatch or hatch with very few weak chicks (19). Several factors affect the hatchability rate during storage, the most important of which is the temperature during storage. The storage temperature should be between 12 and 15°C, while maintaining it as constant as possible. The relative humidity in the storage room should be around 70%. Egg turning also plays an important role, as it is preferable to turn (or reposition) eggs daily to prevent the yolk from sticking to the inner shell (20).

Eggshell coatings composed of nanoparticles, particularly those with antimicrobial properties such as silver nanoparticles (Ag-NPs), can positively impact the hatchability of chicken eggs. Studies have shown that silver nanoparticles, when used to disinfect hatching eggs, can reduce the bacterial load on eggshells, potentially improving hatchability and chick quality (21). Furthermore, some research suggests that certain nanoparticles can improve the immune system of the developing embryo. Research suggests that nanoparticle coatings may also benefit from reducing bacterial loads. Research has shown that silver nanoparticles, in particular, significantly reduce bacterial contamination on eggshells. This is crucial because bacterial infections can negatively impact hatchability. Nanocoatings also contribute to improving egg hatchability by reducing bacterial load. Nanoparticle coatings can also increase hatching rates. Some

studies have reported higher hatching rates in groups treated with silver nanoparticles compared to control groups. Improved hatching quality: Studies indicate that nanoparticle egg coatings can improve chick quality, including increased weight and length, and enhance immune status, making them more resistant to disease. The direct effect of coatings on hatchability is generally due to the coating's role in creating a protective barrier that reduces moisture loss from within the egg and prevents microbial growth.

We conclude from this study that the sixth treatment (CMC + TiO_2) achieved the best results in protecting hatching eggs during storage by increasing hatching efficiency, improving the health of hatched chicks, and maintaining the activity of genes related to muscle growth. The chick weight also increased from 42 g (uncoated eggs) to 47 g (coated eggs), and quality indicators improved.

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