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THE EFFECTS OF IN OVO INJECTION BY CRUDE AND NANO-STEROIDAL EXTRACT OF THE *BACOPA MONNIERI* L. ON EMBRYONIC GROWTH AND SOME HATCHING TESTS

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Abstract

This study was carried out in order to understand the effect of hatching egg injection (In Ovo injection) with the Crude and Nano-Steroidal Extract laboratory prepared of the *Bacopa monnieri* L. in some measurements and embryonic tests about 18 days of embryonic growth, as well as, the hatchability, embryo mortality and the vitality of the hatched chicks. Steroidal compounds were extracted from the plant with HPLC testing to identify some of these compounds and their concentration. 705 hatching eggs from the broiler chicken mothers of the Ross 308 at 0 days were used in this study, while the eggs were divided into five treatments of a141 eggs were allocated for each treatment by (47 egg/replicate) and the experimental treatments were as follows: The First treatment (T1) was negative control treatment without injection, while the second treatment (T2) injects by 0.2 ml of N.S (Normal Saline 0.9%)/egg, which considered as the positive control treatment. The third treatment (T3) injects by 0.2 ml of N.S containing of 100 µg of Nano Chitosan Cs TPP/egg, while the fourth treatment (T4) injects by 0.2 ml of N.S containing 100 µg of steroidal extract /egg. Finally, the fifth treatment (T5) injects by 0.2 ml of N.S containing 100 µg of the Nano-Steroidal Extract on Nano Chitosan Cs TPP/egg. The results of the experiment showed a significant increase ($p \leq 0.01$) in the relative weight of embryos in T5 treatment compared to the control and other treatments at 18 days of embryonic growth. As well as, no significant difference was observed between the treatments in the relative weight of the remaining yolk, while the shell relative weight recorded a significant decrease ($p \leq 0.05$) in the T5 injection treatment compared to T3 and T4. Furthermore, the relative weight of the breast portion achieved a significant increment ($p \leq 0.05$) for embryo treatment T5 compared to negative control treatment T1. Moreover, In the case of eggs hatching traits, it was found that the injection treatments did not effect on the hatchability of fertile eggs nor in the embryo mortality percentage and their stages. Finally, the vitality of the hatched chicks showed a significant improvement ($p \leq 0.01$) for injection treatments T5 compared with other treatments, followed by the injection treatment T4, which recorded a significant difference ($p \leq 0.01$) compared to the negative and positive control treatment. It can be concluded from this experiment, that the injection of hatching eggs (In Ovo injection)to the broiler chicken mothers eggs with the Nano-Steroidal Extract of The *Bacopa monnieri* L. and it Nano Chitosan carrier (Cs NPs) at 100 µg / egg before entering the incubation machine resulted in a significant increase in the embryos relative weight and they breast muscle with 18 days of embryonic growth, as well as, a significant improvement in the vitality of the hatched chicks.

Keywords: Ovo Injection, Nano-Steroidal, Hatching

Introduction

The recent breeds of broiler are characterized by rapid growth and higher performance in the production in the terms of weight and food conversion efficiency than it was in the past, which made them suffer from lack of energy supply and some nutrients during the early stages of growth of chicken embryos within the incubation period of hatching eggs. (Orlov1987) pointing out that, the transition of food additives, such as nutrients, energy, amino acids, vitamins, minerals, and other, from chicken body to the egg about 25-30% and the rest goes into the mother's body. Therefore, the interest in supporting broiler chicken breeds from the early stages of growth and the embryonic development with all the nutrients and substances that effectively contribute to the acquisition of high-weight and vitality chicks. Furthermore, the improvement of hatching rate is very important, which was the purpose of the injection hatching eggs technology (in Ovo injection) that considered as one of the most important technologies that contribute to the production of healthy, high vitality, good health, and distinct productive performance chicks (Naji *et al*., 2007).In this field, the researchers had focused on many nutrients and steroids by injection into hatching eggs with early and late ages of embryonic growth, such as vitamins and amino acids (Al-Asadi, 2006; Abdullatif and Al-Jaaf, 2007; Alshammari, 2012), steroids (Al–Daraji *et al*., 2011), hormones (Kocamis *et al*., 1999; Al-Salhie, 2005), vaccines (Sharma and Burmester, 1982; Sokale *et al*., 2018), extracts (Fahad and Al-shammari 2013; Coskun *et al*., 2017) in addition to Nanomaterials (Beck *et al*., 2015; Patric *et al*., 2016; Hassan, 2018), that give high support to the embryos, which can be obtained a thatched chicks with good quality and high performance later. Phytosterol) are one of the secondary metabolites active compounds, which were found in most plant, especially medicinal plants, including *Bacopa monnirei* L. and have significant effective roles in many medical, pharmaceutical, and breeding and production of poultry, (Subashri and Justin, 2014; Pawar *et al*., 2016). Recently, these compounds have received an extensive attention from several researchers because of their role in the growth and improvement of the muscle mass size for the commercial broiler chickens by improving the level of gene expression in the muscle tissues that responsible for the multiplication and differentiation of muscle cells, which gave an appropriate formal development in the muscle mass (Naji *et al*., 2014). These compounds are also considered to be the basis for the formation of other compounds such as the 17 β-boldenone, and that after being metabolized into the organism's body (De Brabander *et al*., 2004; Scarth *et al*., 2009). The Boldenone consider asone of the anabolic androgenic steroid types that called as hydrogenated Testosteronum unsurpassed (Elks, 2014), and chemically called ά1-testosterone, which is characterized by an active and highly dynamic action in stimulating muscle cells to manufacture protein and muscular construction of broiler chicken and fattening calves (Elmajdoub *et al*., 2016; Neamat-Allah, 2014). Therefore, these natural biological compounds of plant origin and the Boldenonecan be

considered as an alternative to the androgenic hormones of animal origin, such as testosterone, which are still used in several countries of the world to improve the muscle mass of broiler chicken and to obtain high weight rate in the least period time. Numerous studies were proved that, these hormones have adverse negative effects on the animal itself is also reflected in the consumer's health. Finally, this experiment was carried out in order to understand the effect of the hatching egg injection of broiler chickens, ROS 308, with the steroidal compounds extracted of the *Bacopa monnieri* L. in their Crude and Nano images after being loaded on Nano Chitosan (Cs NPs) in some measurements during the embryonic growth stage, the hatchability and embryonic mortality percentage. In addition to the vitality of the hatched chicks one day old.

Materials and Methods

Collection, preparation and extraction of the plant

The *Bacopa monnieri* L. was collected from the banks of Shatt al Arab/Basra, and was prepared for the extraction of the plant extracts laboratory/Biology Department/College of Science for woman/University of Baghdad. The aerial parts of the plant was crushed after the drying process, then the method of alcohol extraction were carried out according to (Pawar *et al*., 2016 and Wagner and Bladt 2009), the maceration operation of the powder has been adopted in this method including 500 weighted powder where placed in a glass bottle, then a 1.5 L of absolute ethanol (99.9%) was added, in addition to a 0.5 L of absolute chloroform (99.4%). The bottle was tightly closed and left in the being watery condition for extract for7 days without being opened, in a dark place and at room temperature. The extract was filtered with filtration paper and vaporized using a rotary evaporator at a temperature of 40-45 °C and the extract leaved at room temperature for 4 consecutive days until it was fully dried as The extract output was 15.5 g thus, a separation and purification of steroid compounds were performed from the rest of the extract by taking 1 g of the extract with the existence of the organic solvent (chloroform)with water by (50:50) using the separator funnel (separation) for a one hour. Moreover, the upper part of the separator funnel was taken, which contained of the steroid compounds and left to dry well for use. The steroids were detected in the extract from the organic layers that have been separated by the separator funnel by conducting a Salkowski reaction test, as (Kiruthiga and Sekar 2014) pointed out. As well as, the quality and quantity of steroids were also estimated using High-performance Liquid Chromatography (HPLC) for the resulting steroids from the separation and purification process of the extract, as mentioned by (Ito *et al*., 2010), which included (Campsterol, Stigmasterol, β-Sitostero, Bacosterol, Ergosterol). For the purpose of obtaining a concentration of 100 µg from the steroidal extract dosages/0.2 ml (N.S) Normal Saline. A 100 µg from the extract were dissolved in a few drops from the organic solvent (DMSO), after making sure of well dissolving, a 10 ml (N.S) has been added to it and was well blended using the heater magnetic stirrer (1000rpm) at 45 m° for 30 minutes To ensure a homogenous and well dissolved solution in N.S. This represents the base solution (stock) from which the required concentration of the solution was prepared by withdraw300 µl from it and dissolving into 29700 µl of the N.S and thus, obtaining a 30 000µl (30 ml),which enough to inject a 150 eggs with a concentration of 100 µg of steroidal extract per 0.2 ml that are injected into one egg.

Preparation of Nano compounds for injection:

The Chitosan powder (German origin) was obtained from a scientific office and Chitosan Nanoparticle (Cs NPs) was prepared for it, in presence of Sodium Tri Poly Phosphate (STPP) compound as a correlation factor with the Chitosan to produce the Chitosan nanoparticles during Inotropic gelation method (Xu and Du, 2003; Dounighi *et al*., 2012; Ghadi *et al*., 2014). In order to prepare what was required for the egg injection, a15 mg of the prepared (Cs NP) has been taken and solved into a 30 ml of N.S. solution (0.9%N.S), then was put into a cold water bath and subjected to ultrasonic for 5 minutes using the prop sonicatre device. The steroidal extract was linked to the Chitosan Nanoparticles compound using a similar approach to what (Lee *et al*., 1998, Yoksan *et al*., 2010, Aiping *et al*., 2006) mentioned with some modification. Steroidal extract was taken and dissolved well in DMSO, then subjected ultrasonic for 5 minutes using the prop sonicatre device to get the steroidal extract to the Nanoscale size, and added by distillation to the Chitosan Nanoparticles solution with the presence of Maleic Anhydride as a connection between them under magnetic movement for two hours at 45 ºC temperature to obtain the resulting component form (CsNPs-Linker-Steroid), which was also subjected for ultrasonic by prop sonicatre device for five minutes.

Description and diagnosing of the prepared Nano compounds

Some measurements have been conducted for the purpose of describing and diagnosing the prepared Nano compounds, including diagnosing their chemical content using Fourier Transform Infrared spectroscopy (FT-IR) for the purpose of identifying new groups that emerged after linking and loading the Nano steroidal with the Nano Chitosan. The other measurement included Nanoparticles average sizes by using the Dynamic Scattering light Intensity (DLS), which also included the test of Scanning Electron Microscopy (SEM) to obtain an image of the prepared Nano compound. Furthermore, the measurement of (FT-IR) was carried out in one of the University of Baghdad laboratories, while the two other measurements were conducted at the Beem Goster Taban Laboratory and the Rastak analysis lab in Tehran/Iran.

Hatching eggs injection (In Ovo injection)

A 705 hatching eggs (sexed eggs) were brought from one of the broiler chicken mothers fields belong to the AL Haditha Company, where the eggs were collected from one field of the Ross 308 mothers at 45 weeks old, by a floor, rearing, and the ratio of males to females in the mothers was 1:6, feeding of diets contained of 15.2% crude protein and 2774 kcal/kg feed energy level. The eggs were divided into five treatments and 141 eggs were allocated for each treatment by (47 egg/replicate) and the experimental treatments were as follows: The First treatment (T1) was a negative control treatment without injection, while the second treatment (T2) injects by 0.2 ml of N.S (Normal Saline 0.9%)/egg, which considered as the positive control treatment. The third treatment (T3) injects by 0.2 ml of N.S containing of 100 µg of Nano Chitosan Cs TPP/egg, while the fourth treatment (T4) injects by 0.2 ml of N.S containing 100 µg of steroidal extract/egg. Finally, thefifth treatment

(T5) injectsby 0.2 ml of N.S containing 100 µg of the Nano-Steroidal Extract on Nano Chitosan Cs TPP/egg. Eggs were injected at the age of a zero day in the air sell region by the injector gun that used in the oil vaccine of 0.2 ml and a needle of 25 mm (Bhanja et al., 2004). After egg candling test and determining the air chamber, the shell was cleaned with sterile alcohol and the needle was pierced from the side of the air chamber after the puncture of the shell by using a mechanical hole and then closed the hole using a nail dyes (Al jaf 2005). the eggs were transferred to the hatchery, which containing of the hatcheries and incubators Belgian-made type Petter Saim Fission,2015 model with a digital display (LED) Programmed as a temperature of 99.4 F° and humidity 67% for the incubator. After 18 days of incubation, the eggs were transferred to the hatchery until hatching, where the temperature was at first day 99.2 F°, and the humidity 87%, then, at the second day was 98.2 F°, H 88%, while the third day was 98.5 F°, H 90%, and at the fourth day itwas 98 F°, H 86%, which was the day when the chicks dried after being hatched.

The studied traits during embryonic growth and after hatching

The eggs candling test was carried out at the age of 18 days of placement in the incubator and before being transferred to the hatchery by one hour, nine eggs were taken from each treatment (3 eggs/rep). The egg was weighted then broken in a petri dish to extract its contents, where the weights are taken of each (the embryo, the remaining yolk, and shell) and calculated as as percentages of the egg's weight of the test (Ibrahim and Abdullatif, 2001). In addition to the calculation of the relative weight of the breast piece after it was separated from the embryo's body with a sharp end convex scissors, moreover, the hatchery rate of the fertile eggs was calculated according to (Naji *et al*., 2009) method. The mortality embryos ratio has been calculated through the calculating of the number of mortality embryos per replicate after test of remaining eggs at the end of the hatching period according to (Khatab *et al*., 1992). As well as, determining the age stages of embryo mortality during the egg incubation period and they hatched, which were divided into three periods according to the stages of embryonic growth as follows: The first was(1-6 days), the second (7-15 days), and the third (16-21 days). Finally, the vitality of the hatched chicks was calculated by turning the chicks over his back and calculate the required time for its standing on his legs according to (Najiet a., 2009) findings.

Results and Discussion

The test results of HPLC technique and after comparing it with the standard showed the presence of steroid compounds (campsterol, Stigmasterol, β-sitosterol) with concentration of 452.01, 200.56, 77.41 µg/ml respectively and with a ratio of 61.91%, 27.48%, 10.60% respectively. As shown in Fig1. A and B. While the results of the spectroscopic diagnoses of the FTIR technique of the Nano-Steroidal compound linked with Chitosan Nanoparticles (Cs NPs-linker-steroid) showed a band absorber at (1745.58) cm^{-1}, which related to Ester bond stretching (O-C = O), the results also showed a presence of band absorber at (3371.5) cm^{-1} back to the acid, hydroxyl group in addition to band appeared at (1654.9) position returns to the Amide group bond frequency(C=O) for the prepared compound.

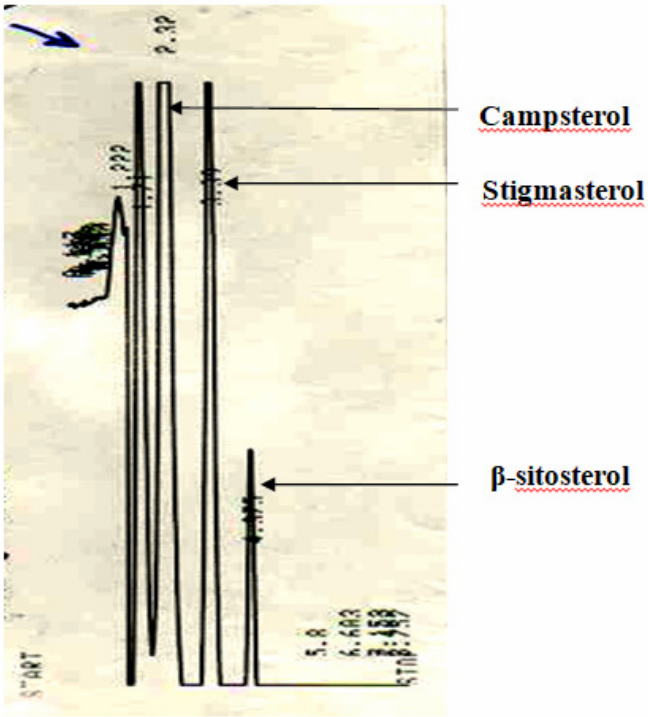


Figure 1-B chromatography for the extract after separation, shows the steroid separation (Stigmasterol , β-sitosterol, Campsterol)

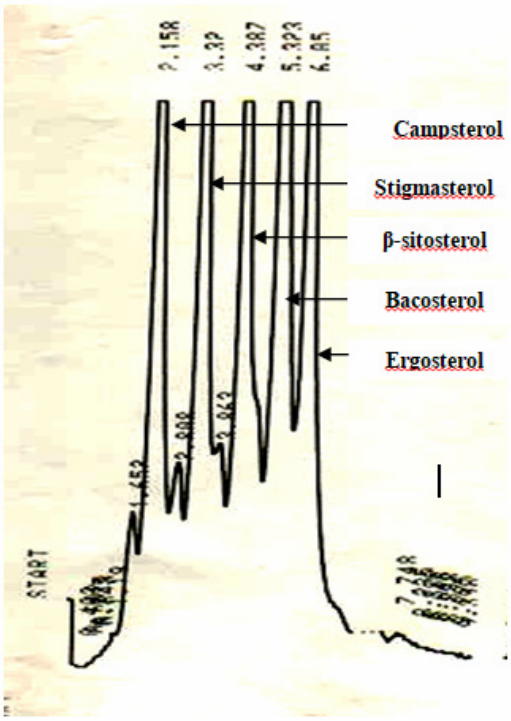


Figure 1-A chromatography For standard Phytosteroids (Campsterol ,Stigmasterol, β-sitosterol, Bacosterol, Ergosterol)

As well as, the appearance of a band in (3481.51) returns to the vibration of the Hydroxyl group (OH) and a band at position (2926.01) cm^{-1} and (2856.58) cm^{-1} returns to the vibration of Aliphatic bond (C-H) as shown figure (2).

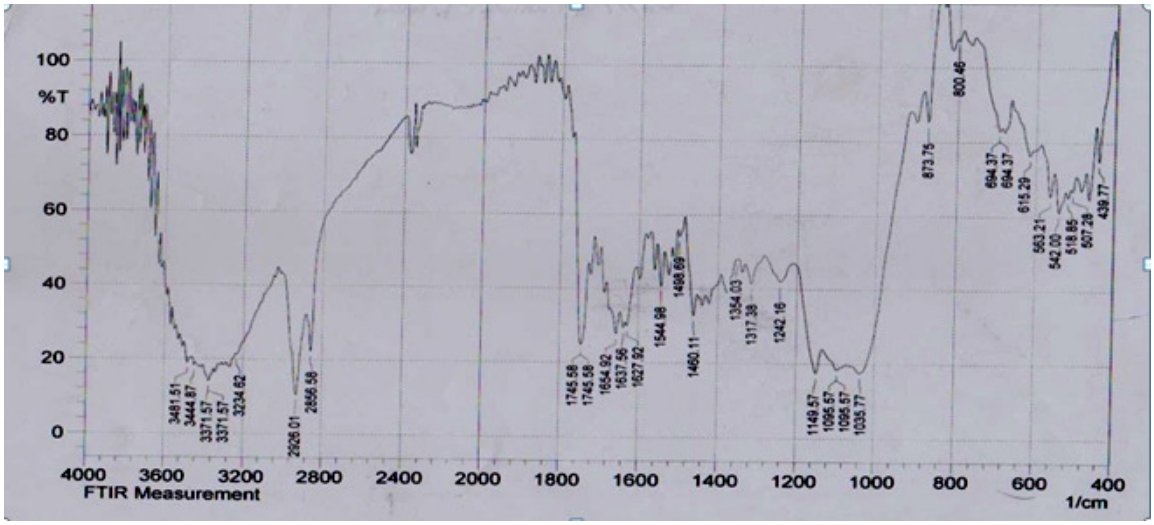
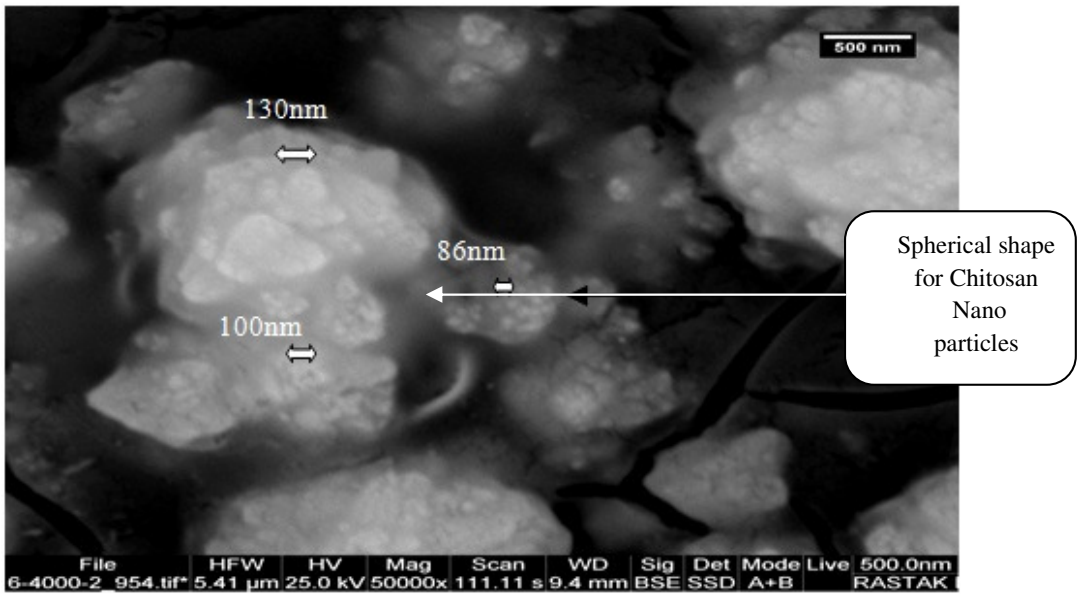


Fig. 2: FT-IR Analysis of the Nano-Steroidal Extract linked with Nano Chitosan with the presence of Maleic anhydride existence

- The size measurement of Nanoparticles (DLS) results showed that the average size of the Chitosan Nanoparticles granules linked with the Nano-Steroidal Extract between them was 137.9 nm and this was agreed with (Kaur *et al*., 2011), while the testing by the Scanning Electron Microscopy (SEM) for the Nano Chitosan after loaded the Nano-Steroidal Extraction it as shown in the Picture (1).



Picture 1 : The Chitosan Nanoparticles which carrier the Nano-Steroidal Extract, according to the SEM test, where shown in spherical form at the 500 nm scale, it was observed that they were the result of the smalls spherical-shaped particle accumulation in a nanometer nm scale

Injection results of the crude and Nano-Steroidal extract in embryonic growth at age 18 days from egg incubation

The injection results that listed in Table 1 showed that the injections treatment T5 had a high significance ($p < 0.01$) in the relative embryo weight compared to the control treatments and the injection treatment T3 and T4. While, The T3 and T4 treatments recorded a significant increase ($p < 0.01$) compared to the PC treatment T2, the control treatments and T3 and T4 treatments did not differ with each other significantly. The injection treatment T5 has a significant decrease ($P < 0.05$) in the relative shell weight compared to T3 and T4 injection treatments, which was not between them and the control treatment T1 and T2 any significant difference. A highly significant ($P<0.05$) was observed in the relative weight of the breast muscle relative to embryo weight at this age of embryonic growth in the injection treatment T5 compared to the NC treatment T1, and there was no significant differences between the treatments T2, T3 and T4.The increasing in the relative embryo weight and the breast muscle in the injection treatments (T5) at this age was a result of the Nano-Steroidal extract effect on the tissues and body muscle cells of the embryo, especially with the presence of Steroidal compounds such as (camp sterol and Stigmasterol and β-sitosterol). This increasing can be explained by being an effective compound, which can get its metabolism inside the body to Anabolic Androgenic Steroid (AAS) such as Boldenone (Ros *et al*., 2007; Poelmans *et al*., 2003; De Brabander *et al*., 2004) that known as ά1-testosterone (Elks, 2014; Morton, 2012; William, 2011), which was naturally considered as AAS.

Table 1 : Effect of In Ovo injection with the Crude and Nano-Steroidal Extract and it Nano compound carrier in embryonic growth at age 18 days of incubation

Treatments	% As percentage of egg weight at testing			Breast muscle relative to embryos weight
	Embryo	yolk	shell	
T1	50.02 ±0.38 ^{bc}	22.01± 0.97	9.80± 0.21 ^{ab}	4.04± 0.42 ^{b}
T2	49.41 ± 0.87 ^{c}	22.45±1.21	9.86±0.01 ^{ab}	4.21±0.37 ^{ab}
T3	51.54 ±0.91 ^{b}	23.13±2.14	9.98± 0.05 ^{a}	4.42± 0.35 ^{ab}
T4	51.77 ± 0.24 ^{b}	21.85±1.11	10.06± 0.04 ^{a}	4.53± 0.15 ^{ab}
T5	53.94±0.10 ^{a}	19.60± 0.47	9.48± 0.25 ^{b}	5.24 ± 0.20 ^{a}
Significant level	**	N.S	*	*

Note 1:- T1, T2, T3, T4. T5 represents the Negative control NC treatment without injection, positive control PC treatment by inject 0.2 ml N.S/egg, inject 0.2 ml of the Nano solution Cs/TPP with concentrations of 100 µg/egg, inject 0.2 ml containing concentration of 100 µg of Steroidal Extract/egg, inject 0.2 ml containing concentration of 100 µg of Steroidal Extract associated with Nano Cs/TPP /egg. At age 0.0 day, respectively.
* * Significant level ($P < 0.01$).
* Significant level ($P < 0.05$).
Arithmetic average ± standard error.
Note 2:- The different lowercase letters within each column indicate of a significant difference.

its work mechanics resulted from the competition on the androgen receptors inside the cell, and its structural effectiveness is to increase nitrogen detention and the protein manufacture, especially when other Androgenic levels decrease (William, 2011).Steroids are lipid soluble so they can penetrate and be implemented through the cell membrane and directly affect the cell nucleus, where the action AAS begins when the outer membrane of the cell penetrates, it will be linked with the androgenic receptors that exists in the cell cytoplasm, then the compound that contain from the receptor that linked with the AAS spreads into the nucleus and effects on the DNA tape within the nucleus where, either changes its genetic expression (Lavery and McEwan, 2005), or activates the processes that send a signal to other cell parts, such as sending mRNA to the ribosomes for protein manufacturing (Cheskis, 2004). The effect of AAS on the muscle mass as in the embryos breast muscle, which previously its relative weight has been taken might occurs, at least in two ways: First, it increases the production of proteins, and the second was that it increases the recovery time by preventing the effects of the stress hormone corticosterone on the muscle tissue so that the muscle destruction decreases significantly (Brodsky *et al*., 1996). The decrease in muscle destruction that occurs naturally due to the existence of AAS, which discourages the work of other steroid hormones called as glucocorticoids hormones that promote muscle destruction (Hickson *et al*., 1990).The presence of these steroidal Nano scale compounds, especially as they are loaded on a bio-Nano compound such as Nano Chitosan, gave it a high dynamic reaction as shown in the results of the T5 treatment. (Hu *et al*. 2008, Donsi *et al*., 2011, Harris *et al*., 2011) pointing out, It can reduce the packaging or loading by Nano particles of Chitosan from loss in the activity of efficient compounds and treatments and thereby maintain their vital effectiveness for longer and at a higher working rate. This increasing of the relative embryo weight in the T5 treatment can be positively reflected in the weight rates of hatching chicks, Therefore, a high final weight rate can be obtained. (Ohta *et al*., 1999) pointed out, that the injections at the early stage of embryonic development will help the embryo to grow, develop , and the formation of its parts in a correct and complete manner, which lead high-energy and high-weight chicks.

Hatching rate and the hatched chicks vitality, in addition to the embryo mortality percentage and their stages:

Table 2 shows the effect of experimental treatments on hatching ratio, the embryo mortality ratio, and the hatched chicks vitality, while Figure 3 shows the age stages of the embryo mortality. The study findings showed that there was no significant difference between the experimental treatments in the hatching ratio of fertile eggs, Furthermore, there was no significant differences in the embryo mortality percentage between the experimental treatments, Although there was an arithmetical reduction for the injection treatment T5.The vitality indicator of the hatched chicks recorded a significant decrease ($P < 0.01$) in the period of the time required for the hatcheries to stand on their legs, after upside down for the injection treatment T5compared to other treatments, followed by the injection treatment T4 that recorded a significant difference ($P < 0.01$) compared to the negative and positive control treatments (T1 and T2), finally, there was no significant difference between the injection treatment (T3) with the (T1, T2 and T4) treatments. Figure (3) showed that there were no significant differences in the embryo mortality ratio according to the three age stages (1-6, 7-14 and 15-21) days of the egg incubation period. Hatching rate was an important economic trait of breeding and poultry production, as it detects the number of chicks that to be obtained from the egg number in incubation, the calculation of the hatching rate in this study showed there were no significant difference was observed between the experimental treatments in terms of this trait. These results present good agreement with (Al-salhi 2012) findings who injected the hatching eggs of Japanese Quail Bird with the Testosterone, Estrogen hormones and vitamin C to understand their effects in some reproductive, physiological, productive and behavior traits.

Table 2 : Effect of In Ovo injection with the Crude and Nano-Steroidal Extract it Nano compound carrier in the hatching rate(%), the hatched chicks vitality(%), and the embryo mortality percentage(%)

Treatments	Hatching rate (%)	Embryo mortality percentage (%)	Hatched chicks vitality (%)
T1	82.15±3.46	18.84±2.34	5.79± 0.85 [a]
T2	80.81±3.52	19.17±2.45	5.57± 0.61 [a]
T3	81.68±4.52	18.34±1.31	4.57±0.35 [ab]
T4	79.85±2.27	19.88±2.89	3.08±0.19 [bc]
T5	82.24±3.60	16.27±2.67	2.18± 0.21 [c]
Significant level	N.S	N.S	**

Note 1:-T1, T2, T3, T4. T5 some variables as mentioned in Table 1 .
**Significant level (P < 0.01).
*Significant level (P < 0.05).
Arithmetic average ± standard error.
The different lowercase letters within each column indicate a significant difference.

The experimental result indicates that there were no significant differences was observed among all the injection treatments in terms of hatching rate (%) for the hatched chicks. The arithmetic decrease in the total embryo mortality ratio, in addition to that calculated during the age stages in which the embryos were died, especially in the first and last stage of embryonic growth in the injection treatment T5 compared to the control treatments. As well as, in the injection treatment T4 at the last stage, which may return to the effect of Steroidal compounds, especially in the Nanoscale size.(Ghosh *et al*., 2011) pointed out that the Steroidal compound (Stigmasterol) extracted from the *Bacopa monnieri* L., that used against one of the cancer disease types(EAS) that occurred in the experimental mice, has led to an increase in the number of its viable cells, thus, the average of survival time has increased and the mortality rate has less than those infected treatments which the Steroidal compound Stigmasterol has not been used. It should be noted that embryo mortality that happened in the first stage of egg incubation were a result of the accumulation of harmful metabolic products that caused by a vital acts, the most important of which is ammonia and lactic acid. Moreover, it may happen due to the insufficient embryo feeding at the early stages, which resulted from the delayed of blood vessel growth on the yolk and the imbalance of the embryo in the breathing process. As well as, the embryo's adhesion on the shell causes the Amniotic fluid dose not surrounded the embryo or may also be caused by embryonic malformation such as nervous system imbalance disruption and skeleton bone's axis (Bellairs and Osmond, 2005). While at the middle stage of embryonic growth (7-14 days), the embryo mortality percentage has been usually little, whether the conditions and constituents of the hatching were in the standard condition. The last stage of embryonic growth (15-21 days) was considered as the most critical stage in the embryonic life, where it has the highest rate of embryo mortality percentage compared to the rest of the critical stages. In which the growth of the embryo is rapidly integrated, where all of body organs grow rapidly and usually two-thirds of the embryo's weight occurs in the last third of the eggs incubation stage and hatching. As the embryo feeding at this stage transferred from the albumen to the feeding on the yolk, which is an important physiological change, the embryo breathing is also converted into aerial respiration by lungs after it was watery respiration by an allantoises bag (Orlov, 1987, Baggott, 2009 and Naji *et al*., 2009).The study results that related with decreasing in the embryo mortality percentage in the injection treatments with Nano-Steroidal extract present a food agreement with (Najiet al., 2014) findings, which pointed out that the use of Phytosterol as nutritional feeding supplements for the broiler chicken has led to increase the broiler chicks survival which treated nutritionally by these effective compounds.

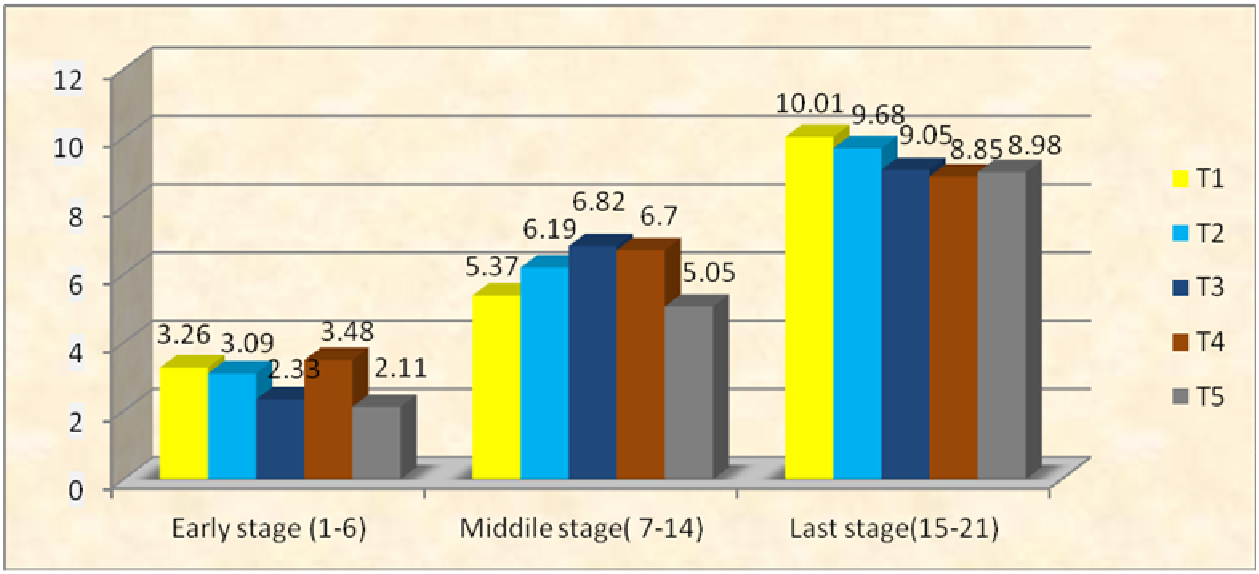


Fig. 3 : Effect of In Ovo injection with the Crude and Nano-Steroidal Extract and it Nano compound carrier in embryo mortality percentage (%) according to age during the incubation period of the eggs

The reason for the a significant improvement in the vitality of the hatched chicks in the injection treatment with the extraction of the Nano-Steroidal extract, as well as the crude Steroidal extract, may be due to the large positive effect of the Steroidal compounds on absorbing the vitamins, which dissolved in the lipid, Carotene (Noakes, 2002; Richelle *et al*., 2004; Korpela *et al*., 2006). In which these are commonly found in the egg yolk, which are used by the embryo in the last growth stage more than in the early stages because it begin to consume the yolk and take the advantage of the necessary nutrients. These vitamins that dissolved in lipid, which plays a very important role in the development, growth of the chick embryos in the correct manner and obtaining chicks with high vitality and activity was vitamin A. The steroidal compounds may be the reason for it well-prepared to the embryo because it positively effects on its absorption by the embryo in large quantities as well as the Carotene in the egg yolk which is represented in the body into two molecules of vitamin A (Donald and Frigg, 2001; Karadas *et al*., 2005).This made the vitamin quantities available to the embryo more higher. (Al-Shammari 2012) pointed out that this vitamin, has played a role in the improving embryonic growth and reducing the mortality percentage, in addition to the increasing of hatching rate, improving the chick's quality in the appearance and vitality, and increase the hatched chicks weight, after injecting the hatching eggs and study its effect on embryonic growth and after hatching.(Wang and Scot 2008) stated that vitamin A has an important and essential role during the natural growth of organs, different compositions, embryonic growth, and thus, have many positive roles during the egg incubation period and after hatching, including the hatched chicks vitality, as well as, its essential and important role in an adult's life. In addition, this may be due to the fact that steroidal compounds of plant origin can be represented by the body after it has been absorbed into other compounds with a similar composition for its chemical composition as Boldenone, which has a similar role to the steroidal hormone's role, such as testosterone, which is a stimulant hormone that gives high activity and vitality of the organism (De Brabander *et al*., 2004), thus improving the hatched chicks vitality.

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