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Effect of In ovo Injection with SeNPs or Clove Oil on Growth Performance of Broiler Chicks

Hamada, M. S. [1]; M. H. Abd-elAziz[2] ; Eman A.Elsaid[3] and Norhan M. Sharshera[1]
[1]Biotechnology Department, Faculty of Agriculture, Damietta University, Egypt
[2]Genetics Department, Faculty of Agriculture, Mansoura University, Egypt
[3]Animal,poultry and fish Department,Faculty of Agriculture ,Damietta University
Corresponding author*: Norhan M. Sharshera Email : normohamed @du.edu.eg

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Abstract:

In-ovo injection with SeNPs or natural antioxidants during the incubation period could boost the growth performance of hatchlings. The objective of this study was to evaluate the effect of in ovo injection of SeNPs or clove oil on the growth performance and immunological and physiological responses of boiler chicks. A total of 240 fertile eggs used in the current experiment were obtained from avian broiler breeder flocks. The eggs were randomly divided into four groups, with 60 eggs per group. The first group consisted of intact, non-injected eggs (negative control, NC), and the second treatment injected into the yolk sac with 0.1 ml saline (positive control, PC.). While the 3rd and 4th treatment were injected with 0.1 ml SeNPs and clove oil. Hatched chicks from each group were randomly assigned to three replicates and reared until 35 d of age The results showed highly significant differences among groups in terms of chicks' weight at day old, growth performance, lymphoid organs, serum total antioxidant, and immunoglobulin G (IgG). Additionally, SeNPs and clove oil had a positive effect on the total bacterial count and pathogenic bacteria compared with the control groups. However, the use of SeNPs or clove oils resulted in non-significant differences in serum total protein, albumin, and globulin levels compared to the control groups.

Furthermore, it can be concluded that in ovo injection of SeNPs and clove oil during the incubation period has a positive effect on broiler chicken weight at hatching and growth performance, as well as on the immunological and antioxidant status of broiler chicks.

INTRODUCTION

In hot climates, the poultry industry is susceptible to many thermally challenged conditions; in particular, the absence of sweat glands in broilers increases the breathing frequency (Hartlova et al., 2002), resulting in reduced feed efficiency and weight gain (Borges et al., 2004). Therefore, there is an urgent need to ameliorate the effects of thermal challenges on poultry. Recently, phytogenic additives rich in aromatic acids and flavonoids, which possess antifungal, antibacterial, antioxidant, and anti-inflammatory properties, have been used to ameliorate the effects of thermal stress in poultry (Oke, 2018; Al-Sultan et al., 2019; Kpomasse et al., 2021; Tokofai et al., 2021) and enhance broiler growth and immunity during periods of heat stress.

In particular, there is growing interest in the use of clove (Syzygium aromaticum) as an effective antioxidant phytogenic additive (Gaikwad et al., 2019) because of its antioxidant properties (Mil-Homens et al., 2012). The bioactive components of clove include eugenyl acetate, eugenol, isoeugenol, caryophyllene,and α-humulene(Jirovetz et al. 2006). In addition, clove oil has a positive effect on reducing serum AST evels in broiler chickens (El-Kholy et al., 2021). The hepatoprotective effect of in ovo essential oil may be due to its rich antioxidant status (Olgun, 2016). Recently, SeNPs is the most extremely stable and soluble elements and rising catalytic qualification and high adsorbing ability (Zhang et al., 2001).

Selenium (Se) is an essential micronutrient needed for growth performance, tissue deposition and antioxidant defense systems in poultry (Surai, 2006). It is a co-factor in some enzyme's systems from which acting as an antioxidant and destroying peroxide radicals (Jacques, 2001).

In view of this, this study aimed to elevate the effect of in ovo injection of SeNPs and clove oil on the post-hatch growth performance and physiological responses of broiler chicks..

Materials and Methods:

Experimental Procedures:

A total of 240 eggs with an average weight of 69 g were obtained from a commercial broiler breeder flock (Avian). Before incubation, the eggs were randomly divided into four treatment groups (60 eggs/treatment). The first group, was intact non-injected eggs (negative control, NC.), and the second treatent injected into the yolk sac with 0.1ml saline (positive control, PC.). While the 3rd and 4th treatment were injected with 0.1 ml SeNPs (20PPb), clove oil (10 µml/egg) as the solvent, respectively. All eggs were normally incubated in a forced draught Laboratory Incubator at the recommended incubation temperature (37.7-37.9ºC) and relative humidity (RH) between 55% and 60% in an automatic incubator. Egg injection was performed on day 10 of embryonic development. The injection site was punctured using a hard and thin stylus, and the test material was injected into the yolk sac of each egg using a graded insulin syringe (1 ml). On the 18th day of incubation, all eggs were transferred to the hatcher and kept until hatching at 37.5°C and 70% RH. Hatched chicks from each group were fed ad libitum on commercial starter (1–20 d) and grower (20 - 35d) diets. The birds were subjected to the same management and vaccination programs as those used in commercial farms.

Slaughter test:

At the end of the experimental period (five weeks of age), six chicks/treatments were randomly selected and slaughtered by severing the carotid arteries and jugular veins. After bleeding, the hot carcass weight and giblet (liver and abdominal fat bad) and lymphoid organ (spleen, bursa of Fabricius, and all thymic lobes) weights were measured. All weights were expressed as percentages of the live body weight.

Biochemical analysis of the blood .

Blood samples were collected from six chicks/treatment in tubes and then centrifuged at 3500 rpm for 20 min, and serum samples were collected and stored at -20oC until analysis. Serum samples were assigned for the determination of total protein (TP), albumin (Alb), globulin (Glob) using available commercial kits. The serum total antioxidant (TAC) and immunoglobulin G (IgG) levels and Immunization and Titration against Newcastle Disease virus (NDV) were determined using ELISA kits.

Bacteriological Examination

One gram of each cecal sample was collected and homogenized from six broilers/group and transferred to the laboratory with minimum delay for examination. They were then transferred under a stream of CO2 into tubes containing 9 mL of sterilized water to examine the total bacterial count on Nutrient Agar, Lactobacilli SP. were aerobically assessed on selective agar MRS. (De-MAN et al., 1960), whereas coliform bacteria on MacConkey agar (MacConkey, 1908) were incubated aerobically at 37º C for 24 h.

Statistical analysis:

Data were statistically analyzed one way analysis of variance using SAS program (SAS,2003) . Differences between means were detected according to Duncan of Ducks in different groups .

The following Statistical model was used :

$Xik = M + Li + eik$

Where :

Xik = An observation,

M = Over all mean ,

Li = Effect of injection materials (i= 1,2,3 and4),

eik = Random error.

Result and Discussion

Performance parameters:

The effects of in ovo injection on the production performance of broiler chicks during the experimental period (1-35 days of age) are shown in Table 1. It is clear from the present results that both in ovo injection with SeNPs and clove oil significantly improved body weight, carcass part yield, and giblets of broiler chicks compared to the control groups. This may be due to the anabolic effect of SeNP and clove oil treatment on muscle building.

A highly significant difference was observed in the initial weight between the different treatment

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Table. 1. Effect of in ovo injection with SeNPs or clove oil on growth performance and carcass characteristics of broiler chicks at 35 days of age

Items	HW (g)	LBW (g)	BWG (g)	Carcass %	Liver %	Spleen%	Thymus%	Bursa%	Abdominal Fat bad%
Control	46.50± 0.5000^{b}	2293± 77.00^{c}	2246± 76.60^{b}	69.54± 0.760^{ab}	2.04± 0.097	0.203± 0.0068^{d}	0.3828± 0.00805^{c}	0.053± 0.0025^{b}	2.21± 0.221^{a}
Saline	46.83± 0.543^{b}	2509± 49.57^{b}	2462± 49.42^{b}	67.56± 1.586^{b}	2.04± 0.171	0.221± 0.0041^{c}	0.4423± 0.02338^{b}	0.064± 0.0017^{ab}	1.84± 0.025^{ab}
SeNPs	48.00± 0.930^{b}	2839± 66.86^{ab}	2791± 67.18^{ab}	71.77± 1.278^{a}	2.47± 0.211	0.266± 0.0067^{b}	0.5432 0.01003^{a}	0.076± 0.0035^{a}	1.52± 0.031^{b}
Clove oil	51.00± 0.894^{a}	3031± 72.65^{a}	2980± 73.01^{a}	72.01± 0.429^{a}	2.16± 0.139	0.288± 0.0029^{a}	0.5333± 0.01126^{a}	0.085± 0.0064^{a}	1.40± 0.048^{b}
P- Value	0.001	<0.001	<0.001	0.033	0.222	<0.001	<0.001	<0.001	<0.001

a-c. Means± standard error of means within a variable with no common subscripts differ significantly (P≤0.05). , HW: chicks weight at day old, LBW, live body weight, BWG: Body weight gain

groups and controls. Additionally, there was a significant increase in the final weight of all treated groups compared to that of the control groups. The injected groups (SeNPs and clove oil) had significantly higher live body weight and body weight gain than the negative and positive control treatments It is clearly noted from the present results that in ovo injection treatments during incubation with SeNPs increased LBW and BW gain of broiler chicks. The beneficial effects of SeNPs may be attributed to its role as antioxidant and biological additives to eggs before incubation. These effects of SeNPs injection may be related, in part, to thymus gland activity and to the positive effects of SeNPs in improving nutrients utilization. The valuable effects of pre incubation injection of SeNPs may be interpreted through its recent definition as an antioxidant. SeNPs, given in ovo, increased number of muscle nuclei of 16 day old chicken embryo, indicating of SeNPs in fast growing organism of fast growing chickens. This finding is in agreement with Brown et al.(1981) who concluded that Se supplementation in rats increased muscle protein breakdown leading to heavier body weights (BW). Nano particles can affect muscle development of chicken embryos and, furthermore, if they can be used for in ovo nutrition as carriers of nutrients e.g. glutamine into muscle cells (Pirsljin et al.,2008).

In chickens, previous reports indicated that there are many beneficial influences of selenium on feed consumption, where increased Se levels increased broiler weight, faster growth, higher yielding of broilers and reduced feed efficiency ratio; body weight, weight gain and the prevention of selenium deficiency symptoms and mortality in poultry compared with untreated group (Cantor et al., 1975; Jianhua et al., 2000 and Singh et al., 2006). Se was sufficient to maintain good performance by the broilers, but additional Se appeared to be necessary to optimize growth (Upton et al., 2008).

On the other hand, the result showed an improvement in the productive performance of broiler chickens due to the present active material in clove (Eugenia caryophyllus) which is considered a digestion stimulating factor, and it had an antibiotic effect against organisms in the digestive canal. Mentioned material caused a greater efficiency in utilization of feed, and led to an improvement in the growth performance (Azadegan et al., 2013). In addition, many studies have reported that clove (Eugenia caryophyllus) was rich in trace minerals which are essential for protein and carbohydrate metabolism, and could improve broiler chickens' performance (AL-Tabari et al., 2018).

xThe results showed a highly significant effect of injection treatments (SeNPs and clove oil) on the relative carcass weight of broiler chicks, liver, abdominal fat, and lymphoid organs.

This result is in agreement with Yuan et al. (2011) who reported that breast muscle were significantly increased as the treated Se level increased in each form of Se. One of the methods to ensure adequate nutrient content in the egg is in ovo administration of nutrients, which increases relative carcass weight and liver and lymphoid organs compared to control groups. It is of interest to observe the high relative weights of the whole breast and pectoralis muscles of broiler chicks (Pirsljin et al., 2008).

Table. 2. Effect of in ovo injection with SeNPs or clove oil on immunoglobulin G, total antioxidant and protein fraction of broiler chicks at 35 days of age.

Items	IgG mg/ml	TAC mmol/L	TP g/dl	Glob. g/dl	Alb. g/dl	A/G ratio
Control	5.18± 0.201[b]	1.58± 0.054[b]	4.14± 0.396	2.29± 0.239	1.86± 0.174	0.82± 0.038[a]
Saline	4.48± 0.204[c]	1.61± 0.048[b]	4.30± 0.311	2.58± 0.233	1.72± 0.142	0.69± 0.056[b]
SeNPs	6.87± 0.213[a]	1.91± .031[a]	3.96± 0.307	2.54± 0.174	1.42± 0.135	0.56± 0.015[bc]
Clove oil	6.31± 0.172[a]	1.78± 0.050[a]	4.83± 0.123	3.01± 0.104	1.82± 0.034	0.61± 0.017[c]
P- value	0.011	<0.001	0.230	0.112	0.113	<0.001

a-c. Means± standard error of means within a variable with no common subscripts differ significantly (P≤0.05). serum total antioxidant (TAC), immunoglobulin G (IgG); total protein (TP), albumin (Alb), globulin (Glob) and albumin/ globulin ratio (A/G ratio).

This result is in agreement with Upton et al. (2008), who reported that the percentage of carcass weight was higher in Selenium Particle (SeP)- treated birds. The thigh yield was higher in SeP-treated birds than in birds from the untreated group. The increase in thigh weight yields appears to reflect improved growth in the SeP-treated broilers. Farhat and Chavez (2001)and Simon and Leclercq (1982) indicated that the selection of broilers for low or high ratios of abdominal fat to body weight resulted in two lines of chickens that differed considerably in carcass lipid content while maintaining similar body weights.

Additionally, a large number of biologically active compounds found in cloves may be responsible for the impulse of the immune system.

The Spleen, thymus, and bursa of Fabricius are important immune organs in animals, and their status is closely associated with immune function. Ravis et al., 1988 and Toghyani et al. (2011) reported that the relative weight of immune organs could be used to evaluate the immune status, and greater weights of immune organs usually represent stronger immune functions to some extent.

The total antioxidant activities were influenced by the NPs and clove oil treatments, as reported in Table 2.The results revealed that SeNP and clove oil treatments significantly increased TAC in broiler serum compared with the control groups. NPs minerals have been act as effective antioxidants and prevent free radical reactive oxygen species (ROS)-generating enzymes, which scavenge excessive (O2-) thus reducing and preventing its harmful effect on cells, in addition to inducing lipid peroxidation in vivo(El-Far et al., 2009), and act as an antioxidant in the thyroid gland and glutathione reductase (GPx). In states of selenium deficiency, normal exposure to H2O2 is cytotoxic and associated with DNA damage, thyroid cancer, and autoimmune thyroiditis(Demelash et al., 2004).

Additionally, in this study, clove oil was applied to broilers and showed higher amounts of antioxidants as scavengers, which diminished the formation of free radicals. These results are in accordance with those of Liu et al. (2015) and Song et al. (2020), who reported that clove volatile oil did not trigger any irritation and demonstrated effective and safe antibacterial activity in vivo. In addition, clove oil has the highest ORAC (oxygen radical absorbance capacity) of all Essential Oils, meaning that it is highly effective in fighting against free radicals. Antioxidants have been shown to provide oxidative defense to the intestines and other organs of developing embryos, protecting them from free radicals that could damage development before hatching (Surai, 1999). Antioxidant protection is an important mechanism in chicken development during hatching (Surai, 2002[a,b]).

Earlier studies have shown that clove can be used as a natural antioxidant in avians (Milind, Deepa, 2011 and Arif et al., 2022). Therefore, the use of antioxidants in developing embryos confers oxidative protection to the intestines and

other organs from free radicals that could impair development before hatching (Surai, 1999). Indeed, antioxidant protection is an important mechanism on chickens' development at hatching time (Surai, 2002[a,b]).

The effects of in ovo injection of SeNPs and clove oil on serum immunoglobulin (IgG) in avian hatched chickens are presented in Table 2. Serum IgG was significantly ($p \leq 0.05$) increased in the groups with SeNPs and clove oil treatments compared to control groups. Plant extracts are rich in flavonoids, which act as antioxidants and therefore enhance immune function (Acamovic and Brooker, 2007). In agreement with the present findings, a previous study has indicated that herbs, as natural antioxidants, enhance the concentration of thyroxin, thereby positively influencing the rate of metabolism.

Plasma Total Protein, Albumin and Globulin

Results in Table 4. In ovo injection with SeNPs or clove oil, there was a significant effect on serum total protein, albumin, or globulin in broiler chicks compared with chicks of control groups. However, alb albumin-to-globulin ratio of chicks was significantly decreased by SeNP and clove oil treatments compared to the control groups.

This finding is consistent with the results of a study conducted by El-Dein et al. (2020). The decrease in the Alb/Glb ratio appeared to be due to an increase in Glb rather than a decrease in Alb. This may reflect a positive increase in immunity through elevation of gamma globulin (El-Said and El-Gogary, 2019).

Antibody production.:

The effect of in ovo injection with either SeNPS or clove oil during embryogenesis on the humoral immunity of broiler chicks against NDV challenge are presented in Fig. 1

NDV was significantly ($p \leq 0.05$) increased in the SeNP and clove oil groups compared to the control groups.

It is clearly shown that the highest significant values for antibody production were attained for chicks of SeNPs treatments compared with control groups on d 35 (Fig.1). Chicks in the control group had the lowest antibody titers.

Supplementation of nano-Se increased both humoral and cellular immunity as measured by antibody titer and gave higher antibody response than that untreated control group (Mohaptra et al., 2014).

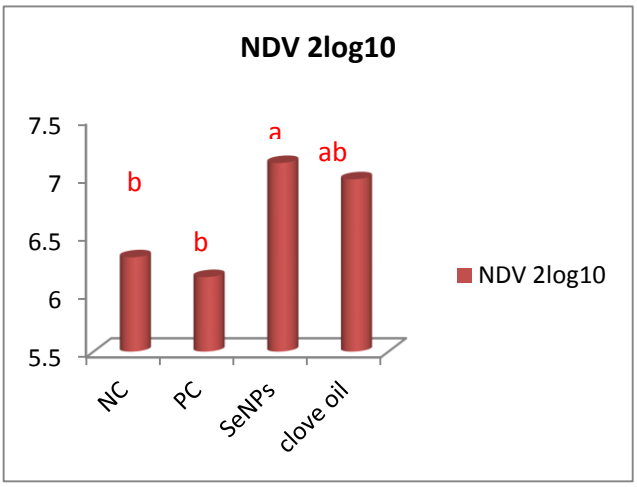

Fig. 1. Effect of in ovo injection with SeNPs and clove oil on humoral immunity of broiler chicks against NDV challenge of broiler chickens

Table 3. shows the populations of total viable bacterial count (cfu × 106), Lactobacillus spp. (cfu × 103), and coliform bacteria (cfu × 103). The results illustrated significant effects of in ovo injection with SeNPs and clove oil on the total bacterial count (cfu × 106) and Lactobacillus spp. (cfu × 103); however, the results revealed that the lowest significant bacterial E. coli count(cfu × 103) was recorded in the injected group compared to other groups.

Injection of SeNPs significantly increased the total bacterial count compared to the control groups. Moreover, the results revealed that clove oil or SeNP injection significantly decreased the count of coliform bacteria, which was more effective in increasing the intestinal count of Lactobacillus spp. (cfu × 103) compared with the control groups. The injected groups showed enhanced positive effects on growth performance, controlling and inhibiting the growth of pathogenic microorganisms. The results revealed that The highest Lactobacillus bacterial count was recorded in the SeNP group, followed by the clove oil group.

It is likewise believed that trace elements like Se increase the population of lactic acid bacteria which produce lactic and acetic acids in the large intestinal tract and which in turn are used as an energy source of intestinal epithelial cell growth that improves nutrient absorption (Eszenyi et al., 2011). Besides its role as an antioxidant and trace element in living systems, few reports have been published regarding antimicrobial potency of nano-selenium (El-Batal et al., 2014). Selenium nanoparticles were also found to be effective antimicrobials against *Pseudomonas* species (Singh et al., 2014).

Table 3. Effect of *in ovo* injection with SeNPs or Clove oil on antimicrobial activity of broiler chicks.

Treat	TBC (cfu x 10^6)	E.Coli (cfu x 10^3)	*Lactobacillus Sp.* (cfu x 10^3)
Control	170.33 ± 16.38^{ab}	79.33 ± 4.15^{b}	34.33 ± 3.27^{c}
Saline	169.50 ± 18.11^{ab}	123.67 ± 11.30^{a}	28.00 ± 2.72^{c}
SeNPs	229.00 ± 39.06^{a}	87.00 ± 4.68^{ab}	159.00 ± 13.70^{a}
Clove oil	101.17 ± 10.97^{b}	47.83 ± 3.39^{c}	97.83 ± 9.07^{b}
P- Value	0.011	0.001	<.0001

a-c. Means± standard error of means within a variable with no common subscripts differ significantly (P≤0.05). TBC: total bacterial count

CONCLUSION

It can be concluded that in ovo injection of SeNPs and clove oil during the incubation period has a positive effect of broiler chicken weight at hatching and body weight gain, as well as on total antioxidant activity, antibody titer and immunoglobulin G of broiler chicks.

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CONFLICTS OF INTEREST:

The authors declare that they have no conflict of interest.

AUTHORS CONTRIBUTION

Hamada, M. S.; M. H. Abd-elAziz; Eman A. Elsaid and Norhan M. Sharshera, developed the concept of the manuscript. All authors checked and confirmed the final revised manuscript.

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الملخص العربى

تأثير حقن أجنة بيض الدجاج بجزيئات السلينيوم النانومترية أو زيت القرنفل على الأداء الانتاجى لكتاكيت اللحم

محمد سعد حماده- محمد حسن عبد العزيز- إيمان أحمد السعيد- نورهان محمد شرشيره.
قسم البيوتكنولوجيا الزراعية- كلية الزراعة، جامعة دمياط، مصر
قسم الوراثة، كلية الزراعة، جامعة المنصورة، مصر
قسم الانتاج الحيوانى والداجنى والسمكى - كلية الزراعة جامعة دمياط

يمكن أن يؤدي حقن أجنة بيض الدجاج باستخدام جزيئات السلينيوم النانومترية أو مضادات الأكسدة الطبيعية خلال فترة التفريخ إلى تعزيز الأداء الانتاجى ومعدلات النمو للكتاكيت الفاقسة. ولذا كان الهدف من هذه الدراسة هو تقييم تأثير حقن البيض بالـ جزيئات السلينيوم النانومترية أو زيت القرنفل على معدلات النمو والاستجابات المناعية والفسيولوجية لكتاكيت اللحم. تم استخدام ٢٤٠ بيضة مخصبة وتم تقسيم البيض بشكل عشوائي إلى أربع مجموعات، بواقع ٦٠ بيضة لكل مجموعة. المجموعة الأولى لم يتم معاملة البيض بأى من مواد الحقن (كنترول سالب، NC). ، والمعاملة الثانية تم حقنها في كيس الصفار بمحلول ملحي ٠.١ مل (كنترول موجب ، PC.). بينما تم حقن المعاملة الثالثة والرابعة بـ ٠.١ مل من جزيئات السلينيوم النانومترية وزيت القرنفل على التوالى. ثم تم توزيع الكتاكيت الفاقسة من كل مجموعة بشكل عشوائي على ثلاث مكررات وتم تربيتها حتى عمر ٣٥ يومًا.

أظهرت النتائج وجود فروق معنوية عالية بين المجموعات من حيث وزن الكتاكيت عند عمر يوم ومعدلات النمو ووزن الأعضاء الليمفاوية ومضادات الأكسدة الكلية في الدم والجلوبيولين المناعي G (IgG). بالإضافة إلى ذلك، كان لـ جزيئات السلينيوم النانومترية وزيت القرنفل تأثير إيجابي على إجمالي عدد البكتيريا والبكتيريا المسببة للأمراض مقارنة بمجموعات التحكم. ومع ذلك، أدى استخدام جزيئات السلينيوم النانومترية أو زيوت القرنفل إلى اختلافات غير معنوية في مستويات البروتين الكلي والألبومين والجلوبيولين في الدم مقارنة بمجموعات التحكم.

علاوة على ذلك، يمكن أن نستنتج أن حقن البيض بـ جزيئات السلينيوم النانومترية وزيت القرنفل خلال فترة التفريخ له تأثير إيجابي على وزن دجاج التسمين عند الفقس وأداء النمو، وكذلك على الحالة المناعية ومضادات الأكسدة لكتاكيت اللحم.