DJAS., Vol. (3) (II) (78-95) (2024)

Damietta Journal of Agricultural Sciences
http://publication.du.edu.eg/journal/ojs302design/index.php/agr/index
ISSN: 2812-5347(Print)- 2812-5355 (Online)

Effect of In ovo injection with selenium nanoparticles and essential oil on related gene expression and relation to physiological responses on broiler chicks

Hamada, M. S. [1]; M. H. Abd-elAziz[2] ; Eman A.Elsaid[3] and Norhan M. Sharshera[1]

[1]Agric-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

ARTICLE INFO

Key words:
In-ovo, Injection, Broiler, gene expression, IGF-1,PPARs

Abstract:

The objective of our study was to elucidate the effect of in-ovo injection of (Selenium Nano Particles (SeNPs), clove oil and nanocapsulated oil on growth efficiency and some physiological markers of avian chicks and gene expression. A six-hundreds of fertile eggs were obtained from Avian broiler breeder herd. All eggs were divided into ten treatments in a (2 *5) factorial design that included the two main chicken strains (Ross 308 or Avain 48) and every main group was divided into five equal treatments of in-ovo injections. First group (B1: without injection), 2nd (B2: was injected with 0.1 ml saline, while third group (B3), 4th (B4) and 5th (B5) treatments were injected with 0.1 ml / egg SeNPs, clove oil and Nanoemulsion oil, respectively. Hatched chicks from each group were split them into five replicates and raised until 35 d of age, and expression patterns for PPARα, PPARγ and IGF-1 genes in tissue liver.

Results showed a highly significant differences between treatments in entries. of chicks' weight at day old and growth performance. Additionally, SeNPs and clove oil had a positive effect on fat deposition and changes in gene expression on lipids of broiler chicks. Significantly differences of gene expression of IGF-1, PPARα and PPARγ genes were recorded between all experimental birds. IGF-1 and fatty acid oxidation affected with SeNPs, clove oil and nanocapsulated oil, nevertheless the activity of IGF-1(insulin growth factor) was enhanced with injection materials. While, the PPARα and PPARγ gene showed a decrease in transcriptional activity in chickens injected with SeNPs, clove oil and nanocapsulated oil.

INTRODUCTION

Nowadays, in-ovo injection is exceedingly used for several purposes, in-ovo injection with immunological material (Sawosz et al., 2012), lift the body weights at day old of avian (Salary et al., 2017), improved immune status (El-Deep et al., 2020); modulation of intestinal development and function (Tako et al., 2004 and El-Said, 2015) by accelerating enteric development in broilers for nutrient absorptive capacity through in-ovo period (Uni and Ferket, 2004; Bhanja et al., 2004; Bakyaraj et al., 2012); increased body weight at marketing (Selim et al., 2012).

Selenium (Se) is an essential micronutrient needed for growth (Li et al., 2019) and currently used as feed additives in poultry (Markovi´ et al., 2018) , vital component of antioxidative proteins and tissue deposition by genetic growth and high metabolic rate of chickens meat (Havenstein et al., 2003 and Tona et al., 2004). In addition, selenium nanoparticles have a positive impact on different organ systems (Raspopov et al., 2011 and Joshi et al., 2011), novel characteristics by good permeability in tissues, low toxicity, elevate antioxidant activity, antimicrobial and anti-carcinogenic properties by rising surface

activity, and rising catalytic qualification and high adsorbing ability (Zhang et al., 2001) and good permeability in tissues and low toxicity (Raspopov et al., 2011 and Joshi et al., 2011). For millennia, people have utilized clove (Syzygium aromaticum), a valuable herb with numerous medical uses, as a food preservation acting as antioxidant additives (Mil-Homens et al., 2012 and Gaikwad et al., 2019). The dried flower buds of the clove tree, an evergreen that thrives in coastal regions and tropical climates, are known as cloves. One of the best sources of eugenol is cloves (Mohammadi et al., 2014).

While some may view genetic research with apprehension, it holds the potential to revolutionize the future of the poultry industry. One particularly useful and safe area of study involves evaluating how nutrition affects gene expression levels in chickens. By examining the gene expression in the muscles and liver, we can quantify these impacts. These investigations can concentrate on nuclear hormone receptors, such as insulin-like growth factor-1 (IGF-1) and Peroxisome Proliferator-Activated Receptors (PPARs) (Wahle et al., 1995, 2003), which are known to be dependent on nutritional status (Heck et al., 2003; Guernec et al., 2004).

In birds, the liver is the primary site of lipogenesis, with a capacity 20 times greater than adipose tissue (based on equal weight) (Griminger, 1986). Pigs, ruminants, and laboratory rodents, on the other hand, primarily synthesize lipids in their adipose tissue (Pearce, 1980). Studies have shown that chicken adipose tissue exhibits negligible lipogenesis (Nir and Lin, 1982). Most of the effects of fatty acid consumption on metabolism are caused by changes in gene expression, which can occur directly or indirectly through nuclear hormone receptors like PPARs (Wahle et al., 2003). PPARs are activated by various fatty acids , fatty acid derivatives, and additional peroxisome proliferators (Dreyer et al., 1992 & Eubank et al., 2001).

PPARs play a critical role in chicken fat cell (adipogenesis) and tissue formation, with PPARγ specifically influencing these processes (**Navidshad & Royan, 2015).** furthermore, Peroxisome Proliferator-Activated Receptor α (PPARα) promotes fatty acid oxidation by upregulating the expression of relevant enzymes **(Bell et al., 1998; Pineda et al., 1999).**

Analyzing the mRNA content of PPARs (PPARα and PPARγ) and IGF-1 in tissues provides valuable insights for formulating optimal fodder for broiler chickens, this is immensely important for the health and nutritional amount of their products **(Abou El-Maaty et al., 2021)**.

Therefore, this study was designed to assess the effects of In-Ovo injection techniques (during embryogenesis) with essential oil, Nano-selenium particles, or Nanoencapsulated oil on live body weight, growth performance, fat deposition, and changes in expression of genes related to lipids and growth in broiler chicks.

3- Materials and Methods:

Ethics Statement*:*

Under the direction of the Agricultural Biotechnology Department of the Faculty of Agriculture at Damietta University in Egypt, this study was conducted at a private farm in kafr-Saad, Damietta Government, Egypt. The Institutional Animal Care and Use Committee of the Faculty of Agriculture, Damietta University, Egypt, approved the experiments, which were conducted in compliance with the ethical requirements of the Committee of Local Experimental Animal Care. Every attempt was made to reduce the animals' suffering.

Sourse of SeNPs, and Nanoemulsion oil:

SeNPs were purchased from Sigma-Aldrich, USA, catalogue (No: 919519). Solid lipid nanoparticles were prepared using ultrasonic-solvent emulsification technique according to Sjostrom Bergenstahl (1992); Siekmann (1996) and Asnawi et al. (2008). Two phases were prepared; oil phase and Nano phase Adel et al. (2015).

3.1. Experimental Design and Egg Incubation:

A total of 600 eggs (.69.91±0.17g) produced by two local chicken strains (Ross 308 and Avain) were collected from commercial broiler breeder flock and were distributed into ten groups of 60 eggs/ group. After wiping the eggs with a clean, dry cloth, each one had its surface washed with a disinfection solution and its shell dried.

On the tenth day after hatch, Yolk Sac was injected in ovo (Bhanja *et al*. 2004). In a factorial study with two main chicken strains {Avain (S1) and Ross 308(S2)} and five equal treatments of in-ovo injections for each main group, the eggs were randomly split into 10 groups. As a negative control, the first group (I1) was left un injected, the second group (I2) received an injection of 0.1 ml saline, and the third, fourth, and fifth groups (I3; I4 and I5) received injections of 0.1 ml / egg SeNPs, clove oil, and Se Nanoemulsion oil, respectively. Following injection, the eggs were placed in the hatcher until they hatched.

3.2. Hatchability and Growth Performance:

On the day of hatch, the hatchability expressed as a percentage of fertile eggs and mortality was recorded. Body weight of chicks were recorded, then the hatched chicks were reared under similar recommended managerial conditions. The broiler chickens was individually weighed at day old and at the end of the experiment body weight gain (BWG) and the amount of feed consumed was recorded (Table 2) and (FCR) feed conversion ratio was calculated.

3.3. Slaughter traits

At 35 d of age, five chicks from each replicate were randomly chosen, weighted, and slaughtered for carcass traits measurements Internal organs (Abdominal fat and liver) were assessed as relative weight.

3.4. Serum Biochemical Indicators

After each group was slaughtered, five blood samples were randomly taken, spun for 15 minutes at 3500 rpm at room temperature to separate the serum, and then stored in 1.5 mL Eppendorf tubes at -20 °C until analysis. Using commercial diagnostic kits from Biodiagnostic Company, Giza, Egypt, the serum concentrations of total protein, globulin, albumin, albumin to globulin ratio, (TG) triglycerides, (Chol.) total cholesterol, (LDL) low density lipoprotein, and (HDL) high density lipoprotein were measured spectrophotometrically in accordance with the methods of Akiba *et al*. (1982) and Taha *et al.* (2019).

Table (1): Composition and chemical analyses of the tested diets fed to broiler chicks from five weeks of age

Ingredients (%)	Starter diets	Growing diets	Calculated analysis (As Fed Basis: NRC, 1994)	
	Control	Control	Metabolizable energy (ME), kcal/kg	3149	3143
Ground yellow corn	61.50	67.20	Crude protein (CP), %	23.06	20.13
Soybean meal (44%CP)	16.00	15.00	Ether extract (EE), %	3.88	3.97
Corn gluten meal (60% CP)	16.50	12.00	Crude fiber (CF), %	2.69	2.68
Sunflower oil	1.00	1.00	Calcium, %	1.20	1.20
Ground limestone	2.00	2.00	Nonphytate P, %	0.45	0.45
Dicalcium phosphate	1.80	1.80	Lysine, %	1.22	1.10
Vitamin and mineral Premix[3]	0.30	0.30	Methionine, %	0.46	0.39
Common salt (NaCl)	0.30	0.30	Methionine + Cystine, %	0.85	0.74
L-Lysine-HCl	0.60	0.40			
Total	100	100			

[1]Premix at 0.30%of the diet supplies the following /kg diet:
Vit.A,1000IU;Vit.D_3,2000IU;Vit.E,10mg;Vit.K,1mg;Vit.B_1,5mg;Vit.B_2,5mg;Vit.B_6,1.5mg;Vit.B_{12},0.01mg;Folic acid,0.35mg;Biotin,0.05mg;Pantothenic acid,10mg;Niacin,30mg;Choline chloride, 250mg;Fe,30mg;Zn, 50mg;Cu, 4mg and Se,0.1mg.

3.5. Gene Expression Examination:

Samples of liver tissue (three birds per treatment) were taken as soon as the birds were killed, frozen in liquid nitrogen, and kept at -80°C for about two days until needed.

In accordance with the manufacturer's recommendations, total RNA was isolated from each sample using the Gene JET RNA Purification Kit (Thermo Scientific, cat. no. K0731) in order to quantify the expression levels of the designated genes.

The concentration and purity of the separated RNA were evaluated with a Thermo Fisher Scientific Inc. NanoDrop spectrophotometer. 18S and 28S rRNA were detected by ethidium bromide staining electrophoresis, where they should show up as distinct, crisp bands. The genomic DNA in the RNA preparations was then extracted using DNase I (RNase-Free).

Step One Applied Biosystems' RevertAidTM First Strand cDNA Synthesis Kit (Thermo Scientific) included a methodology that was followed for cDNA synthesis. The addition of the Ribolock RNase inhibitor came next, and the reaction was then stopped by heating it for five minutes at 70°C. Then, until it was needed, the cDNA was kept at -80°C.

3.5.1. Primer Design:

According to Abou El-Maaty et al. (2021) and based on prior findings, specific primer pairs (forward and reverse) for target genes (PPARα, PPARγ, and IGF-1) and reference genes (β-actin and GAPDH) were generated using Primer-BLAST. These primers were purchased from Invitrogen (Thermo Fisher Scientific) and verified by PCR and gel electrophoresis on cDNA samples.

3.5.2. Quantitative RT-PCR:

Using an Applied Biosystems® Veriti® 96-Well Thermal Cycler and a Master Mix Maxima

SYBR Green qPCR 2x ROX solution, a quantitative 2-step RT-PCR was performed in accordance with the given procedure. The following was the real-time PCR program:

First denaturation: 10 minutes at 95 °C

40 iterations of:

- Denaturation: 15 seconds at 95 °C
- Annealing/extension: 60 °C for 60 seconds (this phase involves data collecting).

The target and reference gene primer specificity was assessed by analyzing the RT-PCR products' melting curves. These curves were run between 55 and 95 degrees Celsius to verify that a single amplicon was amplified. For each gene under test, every sample showed about the same Tm for the target sequence and a single peak in the melting curve (Fig. 1).

Using the 2-ΔΔCT technique, target gene expression patterns were found in liver tissue for both treatment and control samples (Livak and Schmittgen, 2001). Using housekeeping or reference genes as endogenous controls, the expression was standardized. Fold change (FC) values were obtained for the output data in relation to the control (fold relative expression). The prorated expression values were produced through the entry of CT data from target and housekeeping genes into an Excel spreadsheet in Microsoft Office, which contained the equations for the 2-ΔΔCT technique. Using SAS 9 software, the expression of gene data was compared. Duncan's Multiple Range Test was used to set the significance of differences at $P < 0.05$. The statistical program Minitab17 was used to compute a correlation coefficient and a linear regression. The following ranges were analyzed:

*Normalized expression levels in relation to GAPDH and β-actin

* Relative expression of study and performance characteristic genes

3.6. Statistical analysis:

The data were subjected to ANOVA with the generalized linear model (GLM) procedure of SAS software (2004) according to the following model:

$$x_{ijk} = \mu + L_i + P_j + (LP)_{ij} + e_{ijk}$$

Where:

xijk = An observation, μ = Overall mean,

Li = Type of strain (i = 1 and 2),

Pj = Effect of injection (j = 1, 2, 3, 4 and 5),

(LP)ij = effect of interaction between LP (ij = 1,2,3, ….. and 10)

eijk = Random error.

Significance between means were set a statistically at $P \leq 0.05$.

Significance between means of FC values were set a statistically at $P \leq 0.05$ using Duncan's multiple range test (Duncan, 1955).

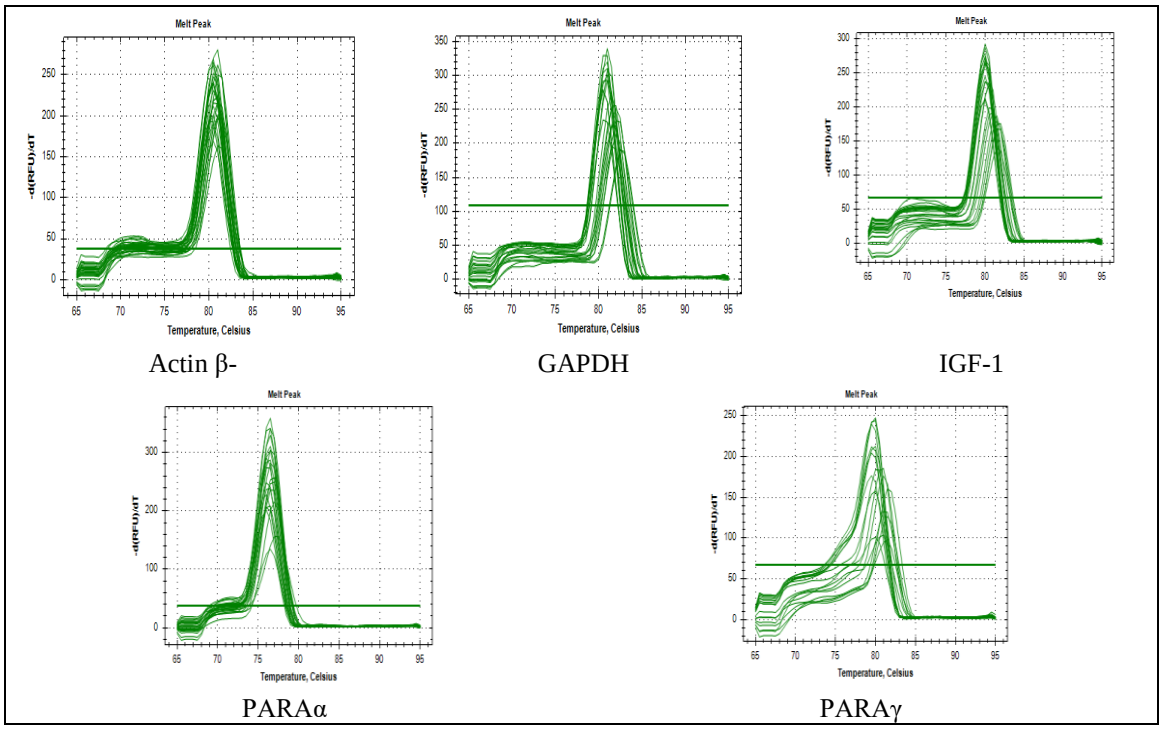

Fig. 1. Melting curves of single amplicon for target and reference genes of interest in this study

4-Results and Discussion

Embryonic Mortality and Hatchability

Results in Fig. 2 illustrated the effect of *in ovo* injection with SeNPs , Clove oil and Nano-emulsion oil on hatchability percentage and across treatments, overall mean percentage hatchability was 85.1,%. The results of the hatchability percentage reflect considerable variations among treatments either strain variation or *in ovo* injection treatments. Results showed that *in ovo* injection with SeNPs treatment and control group recorded the lowest hatchability percent (83.5%) compared to other groups. It has been demonstrated that injecting antioxidants such as selenium (Se) provides oxidative protection against excessive free radicals, which may have a negative impact on hatching in developing embryos (Oke *et al.,* 2021). However, Clove oil and Nanoemulsion oil groups recorded the highest value (86.5 and 87.5%), respectively. As a result, the effects of Nanoemulsion oil in ovo injection treatments showed enhanced values of hatchability percentage compared to other in ovo injection treatments. The improved hatchability observed in clove oil and Nanoemulsion oil groups is in conformity with the observation of Akosile, *et al*. (2023), who reported that clove oil was active in combating heat stress in the later phase of incubation, enhancing hatchability ,decreasing mortality and suggest that the bioactive ingredient of clove (eugenol) was useful in combating the impact of oxidative stress in clove oil treated eggs, and increasing hatchability.

On the other hand, Ross strain showed higher hatchability percentage (86.6%) compared to Avian 48 strain (83.6%). In general, the hatchability percentage results transacting with the effects of strain variation and injection treatments during embryogenesis had different trends. (Bakyaraj et al., 2012) found that in ovo injection with trace elements had no effect on hatchability percent or may reduce it, which is in close agreement with our results.

Productive Performance:

Data in Table. 2. showed insignificant results of egg weight in comparison between all treatments. The effects of *in ovo* injection of SeNPs, clove oil and Nanoemulsion oil on chicks weigh at day old are presented in Table 2. All treated groups had elevated ($p < 0.05$) weight at day old (W0) compared to control groups, and Nanoemulsion oil group recorded the heaviest weight compared to other groups. On the other hand, Avian 48 strain broilers delivered from eggs were significantly heavier than Ross strain broiler weight at day old. It is clearly noted from the present results that injection treatments during embryogenesis in two different strains especially, coating SeNPs with clove oil increased chicks' weight at day old of broiler chicks.

Data in Table. 2. shows insignificant results of egg weight in comparison between all treatments. The effects of in ovo injection of SeNPs, clove oil and Nanoemulsion oil on chicks weigh at day old are presented in Table 2. All treated groups had elevated ($p < 0.05$) weight at day old (W0) compared to control groups, and Nanoemulsion oil group recorded the heaviest weight compared to other groups. These suggests that the bioactive constituents of the additives were beneficial to the chicks during embryogenesis. The current results agree with those of (Akosile, et al. (2023), who confirmed that in ovo injection with clove oil had higher hatchling weight than un injected group.

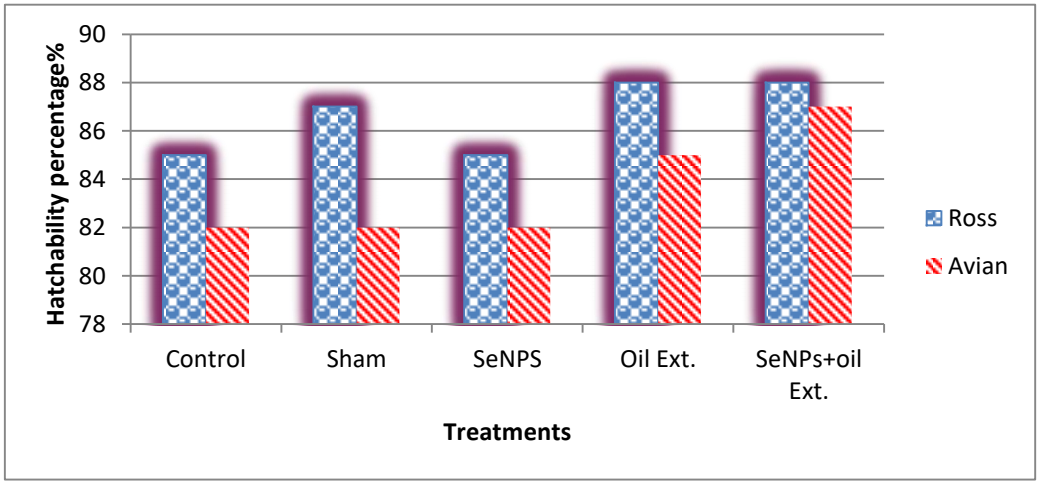

Fig.2. Effect of in ovo injection with SeNPs or Clove oil and Nanoemulsion oil on Hatchability percentage

Table. 2. Effect of *in ovo* injection with SeNPs and/or Clove oil and Nanoemulsion oil on productive performance on two types of broiler chicks.

Treat	Egg weight(g)	W0 (g)	LBW (g)	BWG (g)	FI (g)	FCR
Strain (S)						
Ross St.	69.64	46.60^{b}	2390^{b}	2343^{b}	4238	1.813
Avian St.	70.18	47.67^{a}	2486^{a}	2438^{a}	4320	1.784
SEM	0.24	0.38	27.66	30.11	80.34	0.02
P- Value	0.112	0.050	0.016	0.035	0.447	0.342
Treats (I)						
N. Control	70.20	44.97^{c}	2102.5^{d}	2057.5^{d}	3800^{c}	1.847^{a}
p. Control	69.77	45.00^{c}	2240.0^{c}	2194.7^{c}	4225.0^{b}	1.925^{a}
SeNPs	69.96	47.05^{b}	2420.0^{b}	2372.95^{b}	4361.5^{ab}	1.813^{b}
Clove oil	69.88	48.64^{ab}	2700.0^{a}	2651.37^{a}	4657.5^{a}	1.837^{b}
Emulsion oil	69.74	49.72^{a}	2727.5^{a}	2677.79^{a}	4350.0^{ab}	1.627^{c}
SEM	0.38	0.82	45.23	43.04	130.20	0.017
P- Value	0.825	<0.0001	<0.0001	<0.0001	0.004	0.003
Interaction (S*I)						
S_1*I_1	69.90	44.8	2085	2040.2	3700	1.814
S_1*I_2	69.43	44.8	2190	2145.2	4150	1.935
S_1*I_3	69.65	46.3	2470	2423.7	4523	1.866
S_1*I4	69.68	47.97	2600	2552.03	4515	1.769
S_1*I_5	69.52	49.13	2605	2555.87	4300	1.682
S_2*I_1	70.50	45.13	2120	2074.87	3900	1.880
S_2*I_2	70.10	45.8	2290	2244.2	4300	1.916
S_2*I_3	70.67	47.8	2370	2322.2	4200	1.809
S_2*I_4	70.07	49.3	2800	2750.7	4800	1.745
S_2*I_5	69.97	50.3	2850	2799.7	4400	1.572
SEM	0.54	0.86	61.85	67.32	179.64	0.041
P- Value	0.999	0.968	0.062	0.128	0.532	0.632

a-c. Means± standard error of means, values followed by the same letters are not significantly different at 0.05 level, W0: chicks weight at day old, LBW, live body weight, BWG: Body weight gain, FI: Feed intake and FCR: feed conversion ratio.

On the other hand, Avian 48 strain broilers delivered from eggs showed significantly heavier than Ross strain broiler weight at day old. It is clearly noted from the present results that injection treatments during embryogenesis in two different strains especially, Nanoemulsion oil group increased chicks' weight at day old of broiler chicks. This may be due to in ovo feeding of energy sources is probably to improve hatch weight of broiler as they would supplement the critical needs of these nutrients in the time of piovital period of hatching. Additionally, response to in ovo feeding depend on genetics, incubation conditions, age of breeder hen and egg size (Uni and Ferket, 2004).

Table 2 shows the effect of different types of strains and injection treatments during embryogenesis on productive performance (including LBW, BWG, FI and FCR) of broiler chicks during day old to 35 days of age. The results suggest that Avian 48 strain had increased significant effect on productive performance, except for FI and FCR compared to Ross strain. However, injection treatments during embryogenesis had a positive effect on productive performance of broiler chicks compared to control groups, especially, Nanoemulsion oil group. Overall, LBW and BWG of broiler 6+chickens were affected ($P \leq 0.05$) by both factors (Type strain variation and injection treatments). Also, there was a tendency to improve LBW and BWG in Avian 48 strain treatment, which was injected with SeNPs, clove oil and Nanoemulsion oil compared to control groups. On the same trend, there was a positive effect of injected treatments on FCR exceptionally in Nanoemulsion oil group. Meanwhile, there were no effects between two strains variation on FI and FCR of broiler chicks. The highest FCR was found for control groups, but the lowest for SeNPs+ clove oil. The lowest FCR was in control treatment ($P < 0.05$).

Se nanoparticles enhanced growth performance and decreased FCR, most likely as a result of Se's function in controlling enzymatic systems and metabolism. Se particles are well-known for their antioxidative properties, which aid in preventing intestinal breakouts and improve absorption and digesting processes (Dawood *et al*., 2020; Saleh and Ebeid, 2019). Because selenium is one of the essential elements that can help microbiota complete its activity within the intestine, it can help improve the activity of intestinal microbiota to absorb and digest nutrients, which explains the improved feed utilization (reduced FCR) (Yoon *et al*., 2007).

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 the role of SeNPs in fast growing organism of fast growing chickens. This result is consistent with that of Brown *et al*. (1981), who found that supplementing rats with selenium enhanced the inactivity of their muscles, resulting in larger body weights..Nano particles can affect muscle development of chicken embryos . though, if they can be used for in ovo nutrition as carriers of nutrients e.g. glutamine into muscle cells (Pirsljin *et al*.,2008).

Previous studies on chickens found that selenium has a variety of beneficial effects on feed consumption, including elevated levels of the mineral that increase broiler weight, promote rapid growth, increase yielding, and lower feed efficiency ratios; body weight, weight gain, and the prevention of symptoms and mortality associated with selenium deficiency in poultry when compared to the untreated group (Cantor et al., 1975; Jianhua *et al.*, 2000 and Singh *et al*., 2006). According to Upton *et al*. (2008), selenium was adequate to keep the broilers performing well, although more selenium may be needed to maximize growth.

However, the active ingredient in cloves, Eugenia caryophyllus, which is thought to stimulate digestion and have antibacterial properties against bacteria in the digestive tract, seems to have improved the productive performance of broiler chickens. The substance mentioned improved growth performance and increased feed usage efficiency (Azadegan *et al*., 2013). Furthermore, numerous investigations have found that clove (Eugenia caryophyllus) is a rich source of trace minerals, which are critical for the metabolism of proteins and carbohydrates and may enhance broiler chicken performance (AL-Tabari *et al*., 2018).

Table 2 shows the effect of different types of strain and injection treatments during embryogenesis on productive performance (including LBW, BWG, FI and FCR) of broiler chicks during day old to 35 days

of age. The results suggest that Avian 48 strain had increased significant effect on productive performance, except for FI and FCR compared to Ross strain. However, injection treatments during embryogenesis had a positive effect on productive performance of broiler chicks compared to control groups, especially, Nanoemulsion oil group. Overall, LBW and BWG of broiler chickens were affected (P ≤0.05) by both factors (Type strain variation and injection treatments). Also, there was a tendency to improve LBW and BWG in Avian 48 strain treatment, which was injected with SeNPs, clove oil and Nanoemulsion oil compared to control groups. On the same trend, there were a positive effect of injected treatments on FCR exceptionally in coating SeNPs with clove oil group. Meanwhile, there were no effects between two strain variation on FI and FCR of broiler chicks.The highest FCR was found for control groups, but the lowest for SeNPs+ clove oil. The lowest FCR was in control treatment (P < 0.05).

Carcass weight:

Data in Table 3. illustrated that the relative weight of carcass, abdominal fat and liver insignificantly affected by Ross and Avian 48 strains treatments, but *in ovo* injection with SeNPs, clove oil and Nanoemulsion oil treatments reflected opposite effects. *In ovo* injection treatments had a positive significant effect on improving carcass, abdominal fat and liver relative weight, especially, Nanoemulsion oil group compared to other groups. One of the methods that can improve the relative weights of the liver and at hatch is using clove extracts through in ovo injection (Akosile, *et al*., 2023). According to Akosile *et al*. (2023), this indicates that the in ovo injection of clove extracts stimulated the chicks' digestive systems, improving liver function and raising the pancreatic digestive enzymes.

This result is agreement with Yuan *et al.* (2011) who showed that breast muscle was significantly increased as the treated Se level increased in each form of Se. One of the methods to ensure enough nutrient content in the egg is in ovo administration of nutrients, which raised 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).

This result agrees with Upton et al. (2008), who reported that the percentage of carcass weight was high in Selenium Particle (SeP)- treated birds. Compared to the birds in the untreated group, the SeP-treated birds had a greater thigh yield.

Table. 3. Effect of *in ovo* injection with SeNPs and/or Clove oil and Nanoemulsion oil on Carcass Characteristics on two types of broiler chicks.

Treat	Carcass %	**Liver %**	**Abdominal Fat bad%**
Strain (S)			
Ross St.	68.43	2.09	1.11
Avian 48 St.	68.86	2.23	1.04
SEM	0.493	0.055	0.048
P- Value	0.547	0.066	0.297
Treats (I)			
N. Control	67.93^{ab}	1.98^{c}	1.66^{a}
p. Control	66.48^{b}	2.01^{bc}	1.28^{b}
SeNPs	69.03^{a}	2.25^{ab}	1.02^{c}
Clove oil	69.59^{a}	2.29^{a}	0.75^{d}
Emulsion oil	70.18^{a}	2.27^{ab}	0.66^{d}
SEM	0.970	0.067	0.056
P- Value	0.009	0.020	0.002

a-c. Means± standard error of means, values followed by the same letters are not significantly different at 0.05 level

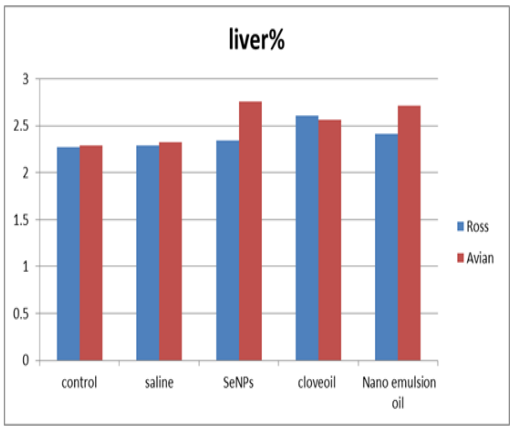

Fig.3. Effect of in ovo injection with SeNPs and/or Clove oil and Nanoemulsion oil on relative weight of liver on two types of broiler chicks.

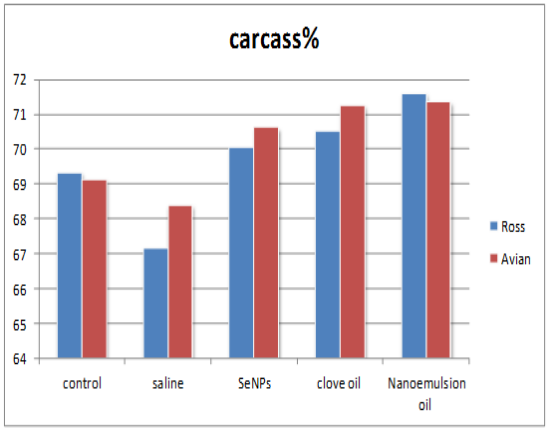

Fig. 4.Effect of in ovo injection with SeNPs and/or Clove oil and Nanoemulsion oil on relative weight of carcass on two types of broiler chicks.

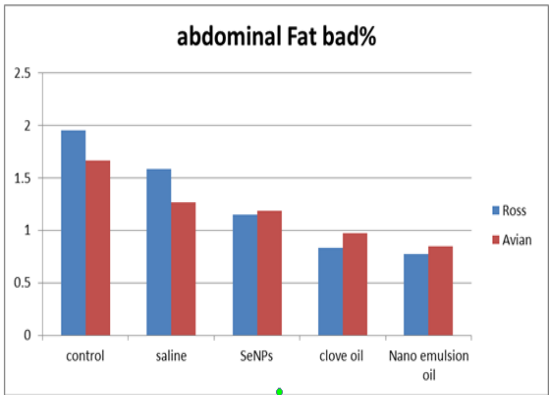

Fig. 5. Effect of in ovo injection with SeNPs and/or Clove oil and Nanoemulsion oil on relative weight of abdominal fat pad on two types of broiler chicks.

It seems that the SeP-treated broilers' enhanced growth is reflected in the rise in thigh weight yields. According to research by Farhat and Chavez (2001) and Simon and Leclercq (1982), selection for broilers based on low or high ratios of belly fat to body weight was evident in two lines of chickens that had significantly different carcass lipid contents but identical body weights.

The effects of different types of strain, injection treatments and their interaction during embryogenesis on blood serum protein and lipids profile of broiler are presented in Table 4. Serum total protein was not affected in different strain treatments, although significant increase in albumin and A/G ratio levels with Avian 48 strain. However, serum globulin level was significantly higher for Ross strain compared by the other strain.

On the other hand, *in ovo* injection treatments during embryogenesis had highly significant effects on albumin, globulin and total protein, and A/G ratio of broiler chicks. Results showed that *in ovo* injection with clove oil and Nanoemulsion oil groups significantly increased serum total protein and globulin as compared to other treatments. Although, clove oil and Nanoemulsion oil groups had significantly decreased serum albumin and A/G ratio. Additionally, serum albumin and A/G ratio of chicks was significantly affected by interaction between various strain and *in ovo* injection treatments. Although, interaction between various strain and *in ovo* injection had no significant effect on serum globulin and total protein .

Of interest, the higher A/G ratio of Avian 48 strain, which reported lower immune response compared by Ross strain. It is known that A/G ratio is a good directrix for immunity in different Avian 48 species, may support the positive impact of clove oil in enhancing immune responses of broiler chicks.

Results in Table 4 clearly show that significant differences exist between strains in serum TG, Chol., HDL and LDL concentration. The results demonstrated that Ross strain significantly decreased serum levels of TG, Chol. and LDL compared to

other strain. However, HDL concentration was significantly elevated in chicks from Ross strain.

On the other hand, results indicated that *in ovo* injection with SeNPs, Clove oil and Nanoemulsion oil showed significant depression in serum levels of TG, Chol. and LDL, however, HDL concentration was significantly elevated in chicks from Nanoemulsion oil followed by clove oil and SeNPs compared to other groups.

Of interest, SeNPs group had a negative effect on lipids profile, which showed significant depression in serum HDL. So, this may explain inconsistent trends in body weight and serum lipids concentration which obtained in the present study.

The results demonstrated that herein no significant differences exist in TG, Chol., HDL and LDL concentration of broilers by interaction between various strain and *in ovo* injection treatments. It is possible that egg treatments can make metabolizable energy to be diverted from embryo chick development to effects associated with nutritious absorption, assimilation and utilization.

Evaluation of Gene expression activity:-

qRT-PCR was used to examine the expression patterns of three genes (PPARα, PPARγ, and IGF-1) in the liver tissue of several experimental groups. To standardize the gene expression levels derived from target genes, two reference genes—β-actin and GAPDH—were utilized. Pearson correlation and regression analysis were performed using Minitab software on the C_T-values of these two reference genes. The relation between C_T-values of β-actin and GAPDH was linear (Fig. 14) by a significant positive correlation (r = 0.67**).

Table. 4. Effect of in ovo injection with SeNPs and/ or Clove oil and Nanoemulsion oil on blood serum protein and lipids profile on two types of broiler chicks.

Treat	TP g/dl	Glob. g/dl	Alb. g/dl	A/G ratio	TG mg/dl	Chol. mg/dl	HDL mg/dl	LDL mg/dl
Strain (S)								
Ross St.	4.78	2.89^{a}	1.90^{b}	0.662^{b}	156.55^{a}	214.12^{a}	58.82^{b}	123.98^{a}
Avian 48 St.	4.69	2.67^{b}	2.02^{a}	0.767^{a}	143.33^{b}	195.03^{b}	66.54^{a}	99.82^{b}
SEM	0.037	0.036	0.0293	0.019	2.1289	2.011	1.037	2.166
P- Value	0.0787	<.0001	0.0047	<.0001	<.0001	<.0001	<.0001	<.0001
Treats (I)								
N. Control	4.62^{bc}	2.55^{c}	2.07^{a}	0.814^{a}	153.30^{a}	211.33^{a}	59.97^{c}	120.70^{a}
p. Control	4.55^{c}	2.64^{c}	1.92^{b}	0.729^{b}	155.08^{a}	210.44^{a}	58.66^{c}	120.77^{a}
SeNPs	4.52^{c}	2.63^{c}	1.89^{b}	0.732^{b}	151.60^{ab}	208.53^{b}	60.88^{b}	117.32^{ab}
Clove oil	5.25^{a}	3.26^{a}	2.01^{ab}	0.613^{c}	147.22^{ab}	200.09^{b}	64.17^{b}	106.48^{ab}
Emulsion oil	7.74^{b}	2.82^{b}	1.92^{b}	0.682^{c}	142.51^{b}	192.47^{b}	69.72^{a}	94.25^{b}
SEM	0.048	0.045	0.042	0.026	2.966	4.960	1.340	6.150
P- Value	0.003	<.0001	0.038	<.0001	0.043	0.0213	0.0001	0.0013

a-c. Means± standard error of means, values followed by the same letters are not significantly different at 0.05 level. TP: total protein, Glob:globulin , ALb:albumin, A/g ratioalbumin to globulin ratio, Triglycerides (TG), total Cholesterol (Chol.), high density lipoprotein (HDL) and low density lipoprotein (LDL)

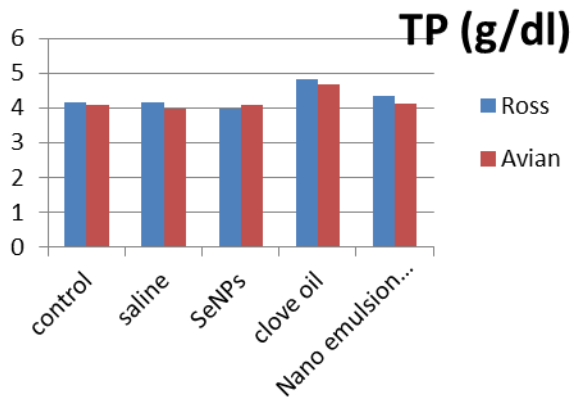

Fig. 6. Effect of in ovo injection with SeNPs and/ or Clove

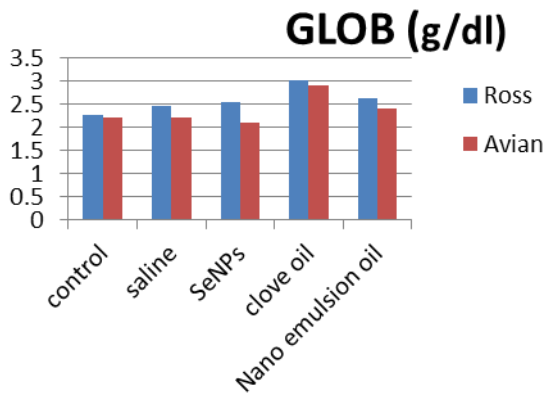

Fig. 7. Effect of in ovo injection with SeNPs and/ or Clove oil

oil and Nanoemulsion oil on total protein on two types of broiler chicks

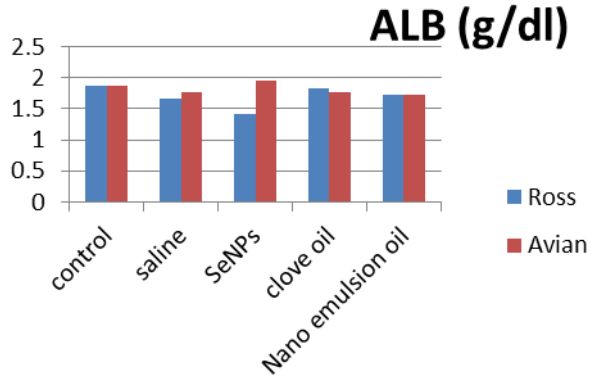

Fig. 8. Effect of in ovo injection with SeNPs and/ or Clove oil and Nanoemulsion oil on albumin on two types of broiler chicks.

and Nanoemulsion oil on globulin on two types of broiler chicks.

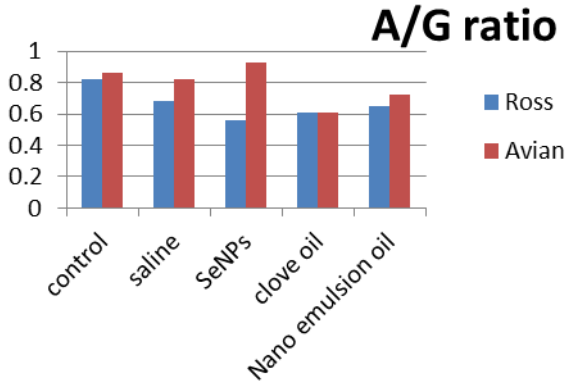

Fig.9. Effect of in ovo injection with SeNPs and/ or Clove oil and Nanoemulsion oil on A/G ratio on two types of broiler chicks.

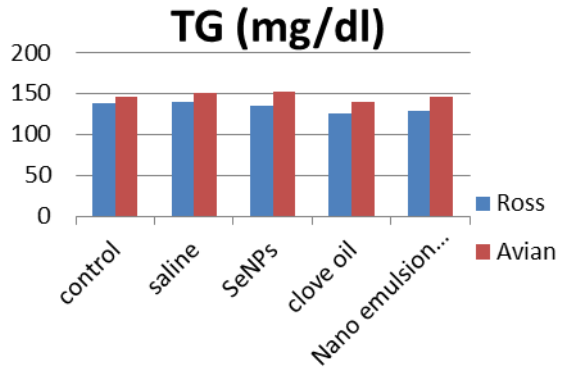

Fig. 10. Effect of in ovo injection with SeNPs and/ or Clove oil and Nanoemulsion oil on Triglycerides on two types of broiler chicks.

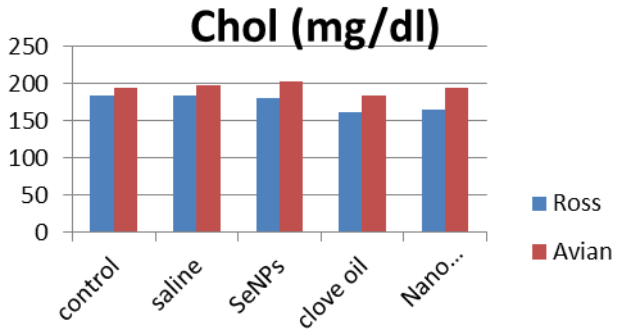

Fig. 11. Effect of in ovo injection with SeNPs and/ or Clove oil and Nanoemulsion oil on total Cholesterol on two types of broiler chicks.

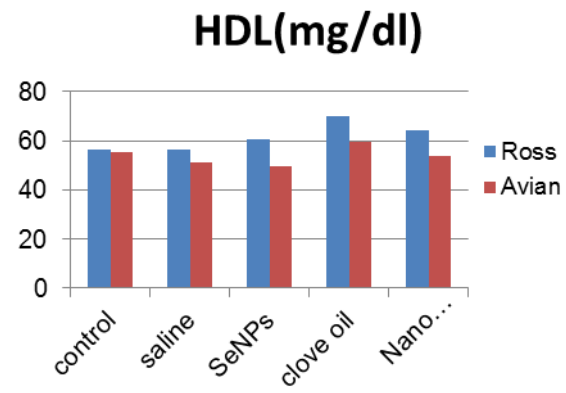

Fig. 12. Effect of in ovo injection with SeNPs and/ or Clove oil and Nanoemulsion oil on high density lipoprotein on two types of broiler chicks.

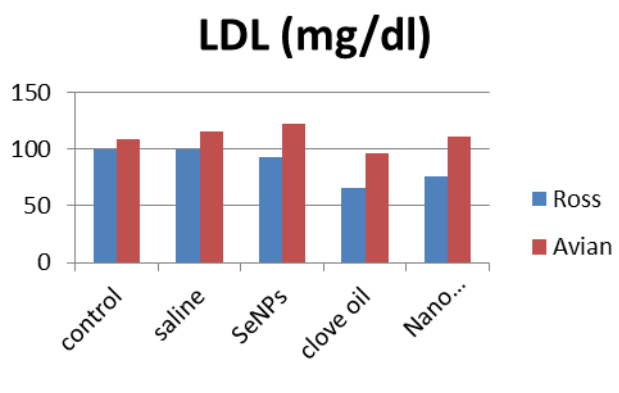

Fig. 13. Effect of in ovo injection with SeNPs and/ or Clove oil and Nanoemulsion oil on low density lipoprotein on two types of broiler chicks.

Based on that, to achieve high measurement accuracy and improve relative expression results reliability, according to Ismail et al. (2022), the C_T-values of a single reference gene in calculation of ΔC_T was change with the average C_T-value from the two reference genes. Where the expression of target genes was normalized with the average C_T-values of the two reference genes

Relative expression patterns of, PPARα, PPARγ and IGF-1 genes in liver tissue, immediately taked after slaughter from Ross 308 and Avian 48 broiler chicken strains injected with treatments of SeNPs, clove oil, nanocapsulated oil, and a non-injected control group, were illustrated in Figure 15.

Significant differences were recorded in the gene expression levels of IGF-1, PPARα, and PPARγ among the experimental birds. This suggests that the activity of growth factor IGF-1 and fatty acid oxidation were influenced by SeNPs, clove oil, and nanocapsulated oil. Interestingly, the efficiency of growth factor IGF-1 was increase by the injection materials, while the PPARα and PPARγ genes showed a decrease in transcriptional action in chickens injected with SeNPs, clove oil, and nanocapsulated oil.

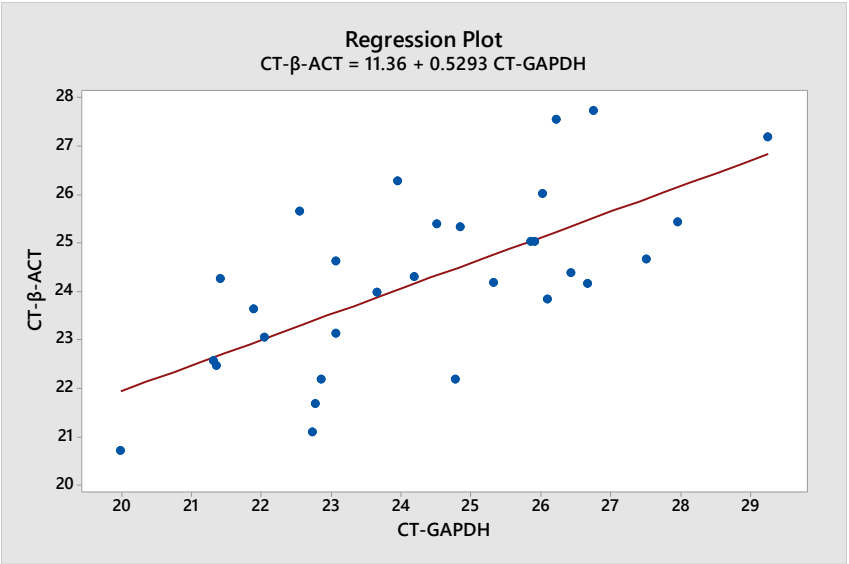

Fig .14. Relationship between C_T values of two reference genes (β-act and GAPDH) used in this study.

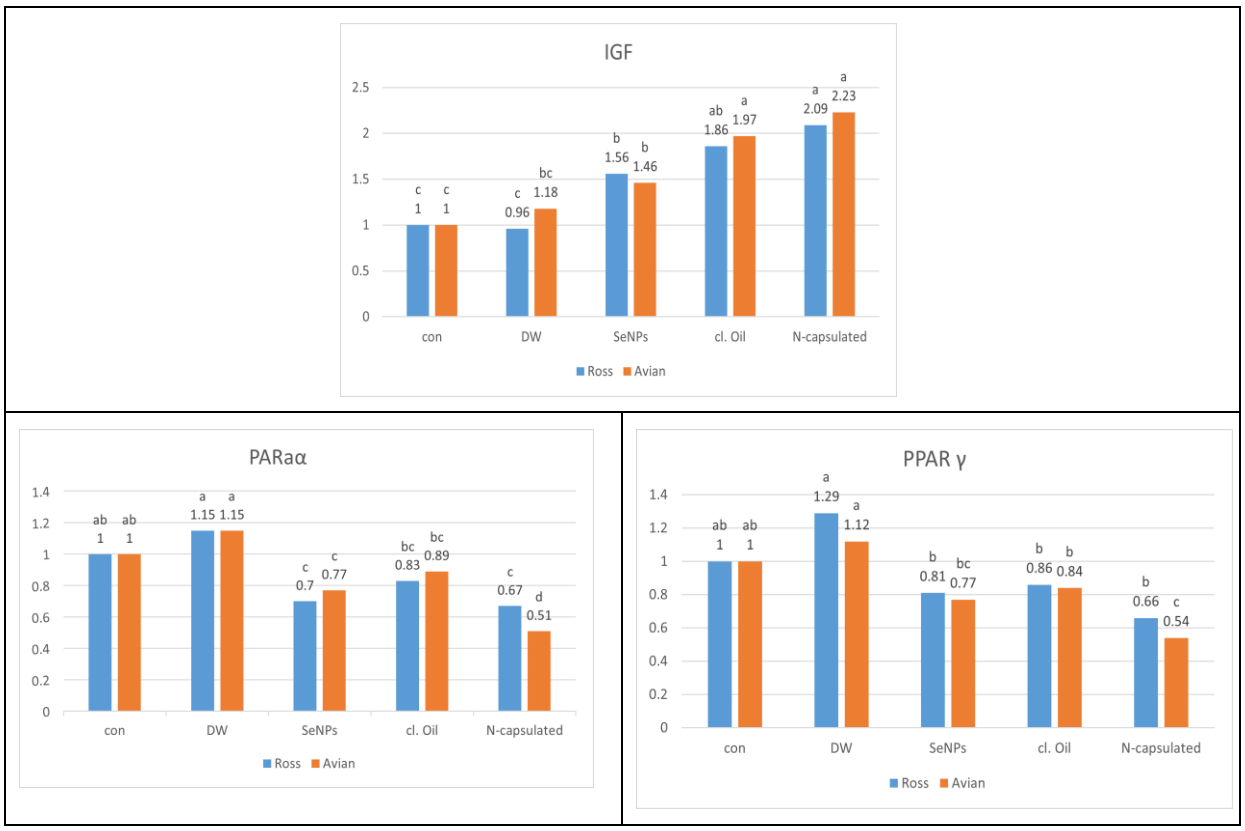

Fig.15:Effect of different experimental treatment on expression on, PPARα ,PPARγ and IGF-1 genes normalized to the expression of GAPDH and β-act reference genes in liver tissue.

Previous studies have shown that (IGF) play a role in cell growth and differentiation, which can be linked to fat storage **(Rechler, 1993).** Additionally, **Beccavin *et al.* (2001)** demonstrated an association between growth rates and IGF-1 activity.

On the other hand, research suggests that dietary fatty acids influence cellular metabolism through changes in gene expression (**Navidshad & Royan, 2016**). They can act as agonists or antagonists for nuclear hormone receptors, upregulating or downregulating specific genes. (PPARs) belong to this receptor family and regulate genes involved in fat metabolism, particularly those related to peroxisomal β-oxidation. PPARs (especially α and γ) control several genes in this process.

Activation of PPARα can indirectly decrease fat deposits by increasing hepatic fatty acid β-oxidation. This is supported by findings that show higher PPARα expression is associated with reduced fat storage in liver cells (**Yoon, 2009**). In contrast, PPARγ plays a key role in fat storage (adipogenesis). It acts as a central gene regulator in adipose tissue, stimulating the expression of genes involved in fat cell formation (**Royan & Bahman, 2016**).

On the other hand, data from Table 5 revealed highly significant positive correlations between Insulin-like growth factor-1 (IGF-1) gene expression levels and mean performance of body weight traits (BWG and LBW) in Ross and Avian 48 strains. Through the post-hatching growth of chickens, this supports the idea of an IGFs-1 stimulatory role and implies a close relationship between IGF-1 and traits indicative of growth efficiency, which may be impacted by nutritional status (Heck et al., 2003; Guernec et al., 2004). These results also demonstrate that the IGF-1 mRNA content in tissues may include valuable information for developing improved broiler chicken feed (Beccavin et al., 2001).

In contrast, the expression level of the IGF-1 gene obtained a highly significant negative correlation with the mean performance of the feed conversion ratio (FCR) trait in Ross and Avian 48 strains. Conversely, a positive correlation was showed between FCR and the expression levels of PPAR-γ and PPARα genes. These findings support the hypothesis proposed by **Yoon *et al.* (2007)** regarding the role of selenium (Se) in improving intestinal

microbiota activity for nutrient digestion and absorption.

Table 5: Correlation coefficients between performance treatment and mean expression levels of IGF-1, PPAR-α, and PPAR-γ genes in ross and Avian 48 broiler chicken strains.

Ross Strain								
Traits	FCR	FI	BWG	LBW	TG	Chol	HDL	LDL
IGF-1	-0.912*	0.715	0.963**	0.964**	-0.919*	-0.885*	0.859**	-0.88*
PPARy	0.849	-0.481	-0.762	-0.763	0.745	0.65	-0.636	0.643
PPARα	0.741	-0.641	-0.815	-0.815	0.699	0.582	-0.611	0.588
Avian 48 strain								
	FCR	FI	BWG	LBW	TG	Chol	HDL	LDL
IGF-1	-0.93*	0.73	0.984**	0.984**	-0.489	-0.39	0.387	-0.38
PPARy	0.945*	-0.298	-0.743	-0.744	0.209	0.009	-0.101	0.026
PPARα	0.93*	-0.206	-0.684	-0.685	0.14	-0.069	-0.031	-0.05

* and ** significant correlation values at 0.05 and 0.01 probability levels, respectively.
FCR: feed conversion ratio, FI: Feed intake, BWG: Body weight gain, LBW: live body weight, TG: Triglycerides, Chol: total Cholesterol, HDL: high density lipoprotein, LDL: low density lipoprotein.
IGF-1, PPARα and PPARy: expression levels for IGF-1, PPAR-α and PPAR -γ genes

The Ross strain uniquely exhibited a significant negative correlation between the expression level of the IGF-1 gene and mean performance of total cholesterol (Chol), low-density lipoprotein (LDL), and Triglycerides (TG) traits. Conversely, it showed a significant positive correlation between the expression level of the IGF-1 gene and the mean performance of the HDL trait. In contrast, the Avian 48 strain exhibited similar trends, but with insignificant correlations. Indicating that the IGF1 gene also plays critical roles in stimulating lipogenesis **(Accili *et al.*, 1996).**

However, additional research has shown that PPAR genes are crucial for the metabolic syndrome and overall health of living things, including tissue regeneration, differentiation, lipid metabolism, and immunological response (Kadivar et al., 2016). Where PPAR-γ gene is known play a critical role in initiating adipocyte differentiation, leading to increased fat deposits (**Royan *et al.*, 2011**). While, PPAR-α gene is play a critical role in regulating lipids homeostasis (**Yessoufou *et al.*, 2009**). Where higher PPAR-α gene expression is associated with reduced lipid deposition in hepatocytes (**Yoon, 2009**). **Royan *et al.* (2011)** also reported PPAR-γ gene upregulation in the adipose tissue of broilers fed oil compared to birds fed a base diet. Similarly, selenium supplementation upregulates PPAR-γ gene expression **(Modarres *et al.*, 2018).**

However, our results in Table 5 showed a positive but statistically insignificant correlation in the Ross strain between Chol, LDL and TG with PPAR-γ and PPAR-α gene expression. Conversely, an insignificant negative correlation was observed with high-density lipoprotein (HDL). While, in the Avian 48 strain, these traits exhibited a poor correlation with PPAR-γ and PPAR-α gene expression. These findings suggest unclear relationships between PPARs genes activation and the studied performance traits, despite many studies confirming the crucial roles of PPARs genes in chicken fat cell (adipocyte) development and tissue formation. Therefore, further research is need to clarify the specific mechanisms underlying these relationships.

CONCLUSION

This study suggests that in-ovo injection of broiler chick with selenium nanoparticles (SeNPs) and essential oil can affect their growth performance and some physiological responses. We observed significant increases in body weight and insulin growth factor-1 (IGF-1) expression. Additionally, there was an indication of improved intestinal microbiota activity, which likely led to enhanced nutrient digestion and absorption. These findings have potential economic importance.

References:

Abo-El-Maaty, Hayam,. M. A.; Abd El-Aziz. M. H.; El-Diasty .M.Z. and El-Said .E. A. (2021). Effect of dietary Rocket seeds meal without or with nano-chitosan on performance, some biochemical parameters and related gene expression to growth and lipid profile in broiler chicks. Egyptian J. Nutrition and Feeds (2021), 24(1):139-155

Accili, D; Drago, J;Lee, E.J; Johnson, M.D;Cool, M.H, Salvatore, P.; Asico, L.D; Jose, P.A; Taylor, S.I and Westphal, H (1996) Early neonatal death in mice homozygous for a null allele of the insulin receptor gene. Nat Genet 12:106–109

Adel, M., W. Atwa, M. Hassan, N. Salem, D. S. Farghaly and S. Ibrahim, (2015). Biological activity and field persistence of Pelargonium graveolens (Geraniales: Geraniaceae) loaded solid lipid nanoparticles (SLNs) on Phthorimaea operculella (Zeller) (PTM) (Lepidoptera:Gelechiidae). Int. J. Sci. Res. 4, 514–520.

Akiba Y.; Jensen, L.S. and Lilburn, M.S. (1982). Effect of Estrogen Implants on Hepatic Lipid Deposition in Chicks Fed Different Isonitrogenous and Isocaloric Diets.The Journal of Nutrition Volume 112, Issue 1, January 1982, Pages 189-196

Akosile, O. A.; Majekodunmi, B. C.; Sogunle, O. M.; Baloyi, J. J.;Fushai, F.; Bhebhe, E. and Oke, O. E. (2023) .Research Note: Responses of Broiler Chickens to In Ovo Feeding with Clove and Cinnamon Extract under Hot-Humid Environments.https://doi.org/10.1016/j.psj.2022.102391. Poultry Science 0032-5791(22)00685-X

AL-Tabari,Afrah. S.; Al-Zuhairi, Zahira A. and Abdulrazzaq ,Mahasen.(2018).Study The Effect Of Adding Aqueous Extract Of Clove (Eugenia Caryophyllus) To Drinking Water In Productivity And Physiological Efficiency Of Broiler Chicken.Dec 2018Basrah Journal of veterinary Research 17(1):165-175.DOI: 10.33762/bvetr. 2018.144949

Asnawi, S., Abd. Aziz, A., R.Khamis, A. K. (2008). Formulation of geranium oil loaded solid lipid nanoparticles for mosquito repellent application. Jor Journal of Chemical and Natural Resources Engineering. 2, 90-99.

Azadegan, M.; Hassanabadi, A.; Nassiri, H. and Kermanshahi, H. (2013). Supplementation of clove essential oils and probiotic to the broilers diet on performance, carcass traits and blood components.Iranian Journal Applied.Animal Science, 4(1): 117-122. Available at: http://ijas.iaurasht.ac.ir/article_513730.

Bakyaraj, S.; Bhanja, S.K.; Majumdar, S. and Dash, B. (2012) Modulation of post-hatch growth and immunity through in ovo supplemented nutrients in broiler chickens.
J. Sci. Food Agric., 92: 313-320.

Beccavin, C.; Chevalier, B.; Cogburn, L.A.; Simon, J. and Duclos, M.J. (2001). Insulin-like growth factors and body growth in chickens divergently selected for high or low growth rate. Journal of Endocrinology, 168: 297–306.

Bell, A.R.; Savory, R.; Horley, N.J.; Choudhury, A.I.; Dickins, T.J.; Gray, A.M., and Salter, D.R. (1998). Molecular basis of non-responsiveness to peroxisome proliferators: The guinea-pig PPAR alpha is functional and mediates peroxisome proliferator-induced hypolipidaemia. Biochem. J., 332, 689–693.

Bhanja, S.K.; Mandal, A.B. and Johri, T.S. (2004): Standardizationof injection site, needle length, embryonic ageand concentration of amino acids for *in ovo*injection inbroiler breeder eggs. Indian Journal of Poultry Science,39, 105–111.

Brown, J. G.; P. C. Bates; M. A. Holliday and D. J. Millward (1981). Thyroid hormone and muscle protein hypophysectomized rats. Biochem. J., 194: 771-782.

Cantor, A. H.; M. L. Langevin; T. Noguchi and M. L. Scott (1975). Efficacy of selenium in selenium compounds and feedstuffs for prevention of pancreatic fibrosis in chicks. J. Nutr., 105:106-111.

Dawood, M. A. O.; Zommara, M.; Eweedah, N. M.; Helal, A. I.; and AboelDarag, M. A. (2020). The potential role of nano-selenium and vitamin C on the performances of Nile tilapia (Oreochromis niloticus). Environmental Science and Pollution Research, 27, 9843–9852. https:// doi.org/10.1007/s1135 6-020-07651 -5

Dreyer, C.; Krey, G.; Keller, H.; Givel, F.; Helftenbein, G. and Wahli, W. (1992).Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. *Cell* **1992**, *68*, 879–887.

El-Deep, M. H.; Amber, Kh. A.; Salwa, Elgendy; Dawood ,M. A. O. and Zidan A. (2020).In ovo injection of nano-selenium spheres mitigates the hatchability, histopathology image and immune response of hatched chicks *J Anim Physiol Anim Nutr*.;104:1392–1400

El-Said, (2015). Influence of in-ovo injection (feed-ing) with Nano particles Selenium (SeNPs) on growth performance, carcass yield and immune status of broiler chickens. Ph.D. Thesis. Fac. Agric., Damietta Univ., Egypt, pp. 48-102.

Eubank, D.W.; Duplus, E.; Williams, S.C.; Forest, C. and Beale, E.G.(2001). Peroxisome proliferatoractivated receptor γ and chicken ovalbumin upstream promoter transcription factor II negatively regulate the phosphoenolpyruvate carboxykinase promoter via a common element. *J. Biol. Chem*. *276*, 30561–30569.

Farhat, A. and E. R. Chavez (2001). Metabolic studies on lean and fat pekin ducks selected for breast muscle thickness measured by ultrasound scanning. Poult. Sci., 80: 585–591.

Farhat, A., & Chavez, E.R. (2001) Metabolic studies on lean and fat Pekin ducks selected for breast muscle thickness using ultrasound scanning. Poultry Sci., vol. 80, pp.585-591.

Gaikwad, D.S.; Fulpagare, Y.G.; Bhoite, U.Y.; Deokar, D.K, and Nimablkar CA (2019). Effect of dietary supplementation of ginger and cinnamon on growth performance and economics of broiler production. International Journal of Current Microbiology and Applied Science, 8(3): 1849-1857.

Griminger, P. (1986).Lipid Metabolism. In *Avian Physiology*, 4th ed.; Sturkie, P.D., Ed.; Springer-Verlag: New York, NY, USA; pp. 345–358.

Guernec, A.; Chevalier, B. and Duclos, M.J. (2004). Nutrient supply enhances both IGF-I and MSTN mRNA levels in chicken skeletal muscle. Domastic Animal Endocrinology, (26): 143-154.

Havenstein, B.; P. R. Ferket and M. A. Qureshi (2003). Carcass composition and yield of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci., 82: 1509-1518.

Heck, A.; Metayer, S.; Onagbesan, O.M. and Williams, J. (2003). mRNA expression of components of the IGF system an GH and insulin receptors in ovaries of broiler breeder hens fed ad libitum or restricted from 4 to 16 weeks of age. Domastic Animal Endocrinology, (25): 287-294.

Ismail, Lamiaa, M.; Soliman, Magda. I, Abd El-Aziz ,M. H, Abdel-Aziz, Heba M.M.(2022).Impact of silica ions and nano silica on growth and productivity of pea plants under salinity stress .J.Plants 11 (4), 494

Jianhua, H.; A. Ohtaukawa and K. Hayashi (2000). selenium influences growth via thyroid hormones status in broiler chickens. Br. J. Nutr., 84: 727-732.

Joshi, U.; Raut, P.D.; Agrawal, S.K.; Patra, P.K.; Maheshwari, B.K. and Apurb, M.(2011). Evaluation of serum selenium level in patients with uncomplicated diabetes mellitus, Raipur, India. J Clin Diagn Res 2011;5:70-3.

Kadivar, A.; Khoei, H. H.; Hassanpour, H.; Ghanaei, H.; Golestanfar, A.; Mehraban, H.; Davoodian, N, and Tafti R.D.(2016).Peroxisome proliferator-activated receptors (PPARα, PPARγ and PPARβ/δ) gene expression profile on ram spermatozoa and their relation to the sperm motility.Veterinary research forum 7 (1), 27

Livak, K.J. and Schmittgen, T.D. (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-ΔΔCT Method. Methods, 25(4): 402-408

Markovic, R.; Ciric, J.; Drljacic, A.; šefer, D. and Starcevic, M.(2018). Corrigendum to "The effects of dietary selenium-yeast level on glutathione peroxidase activity, tissue Selenium content, growth performance, and carcass and meat quality of broilers" Poult. Sci. 2018;97:2618.

Mil-Homens, D.; Bernardes, N. and Fialho A. M.(2012). The antibacterial properties of docosahexaenoic omega-3 fatty acid against the cystic fibrosis multiresistant pathogen *Burkholderia cenocepacia*. FEMS Microbiol. Lett. 328:61–69. doi: 10.1111/j.1574-6968.2011.02476.x

Modarres, Sh. Z.; Heidar, Z.; Foroozanfard, F. ; Rahmati, Z.; Aghadavod, E. and Asemi, Z. (2018). Biol Trace Elem Res 183:218–225 DOI 10.1007/s12011-017-1148-2.

Mohammadi, Z.; Ghazanfari, S. and Morad, M. A. (2014).Effect of supplementing clove essential oil to the diet on microflora population, intestinal morphology, blood parameters and performance of broilers. Europ.Poult.Sci., 78. 11 page. DOI: 10.1399/eps.2014.51.

Navidshad B, Royan M. (2016).Peroxisome Proliferator-Activated Receptor Alpha (PPARα), a Key Regulator of Lipid Metabolism in Avians. Crit Rev Eukaryot Gene Expr. 2016;26(4):303-308. doi: 10.1615/CritRevEukaryotGeneExpr.2016016665. PMID: 27910744.

Navidshad, B. and Royan, M. (2015). Ligands and Regulatory Modes of Peroxisome ProliferatorActivated Receptor Gamma (PPARγ) in Avians. Crit Rev Eukaryot Gene Expr., 25(4):287-292

Nir, I. and Lin, H. (1982).The skeleton, an important site of lipogenesis in the chick. *Ann. Nutr. Metab*., *26*, 100–105.

Oke, O.E.; Oyelola, O.B.; Iyasere, O.S.; Njoku, C.P.; Oso, A.O.; Oso, O.M.; Fatoki, S.T.; Bankole, K.O.; Jimoh, I.O.; Sybill, N.I.; Awodipe, H.O.; Adegbite, H.O.; Rahman, S.A. and Daramola, J.O .(2021). In ovo injection of black cumin (Nigella sativa) extract on hatching and post hatch performance of thermally challenged broiler

chickens during incubation. Poultry Science 100 (3), 100831.

Pearce, J. A.(1980). comparison of the response of hepatic enzyme activity in domestic fowl (Gallus domesticus) and the rat (Rattus norvegicus) to the feeding of diets containing large proportions of glucose and fructose. *Comp. Biochem. Physiol*. **1980**, *651B*, 423–426.

Pineda Torra, I.; Gervois, P. and Staels, B. (1999). Peroxisome proliferator activated receptor alpha in metabolic disease, inflammation, atherosclerosis and aging. Curr. Opin. Lipidol., 10: 151–159

Pirsljin, J.; S. Milinkovic-Tur; B. Beer Ljubic and M. Zdelar-Tuk (2008). The effect of organic selenium supplementation on antioxidative characteristics and lipid peroxidation in chicken blood during fattening and after fasting. Vet. Arhiv., 78: 187196.

Raspopov, R. V., Trushina, É. N., Gmoshinskiĭ, I. V., and Khotimchenko, S. A. (2011). Bioavailability of nanoparticles of ferric oxide when used in nutrition. Experimental results in rats. Vopr. Pitan. 80, 25–30

Rechler MM. Insulin-like growth factor binding proteins. Vitam Horm. 1993;47:1-114. doi: 10.1016/s0083-6729(08)60444-6. PMID: 7680510.

Royan, M.; Goh, Y. M.; Othman, F.; Sazili, A. Q. and Navidshad, B.(2011). .Effects of Conjugated Linoleic Acid, Fish Oil and Soybean Oil on PPARs (α & γ) mRNA Expression in Broiler Chickens and Their Relation to Body Fat Deposits.5*Int. J. Mol. Sci.* 2011, *12*. ISSN 1422-0067 www.mdpi.com/journal/ijms

Royan, Maryam and Navidshad, Bahman(2016). Peroxisome proliferator-activated receptor gamma (PPARγ), a key regulatory gene of lipid metabolism in chicken. World's Poultry Science Journal, 72: 773-784. DO - 10.1017/S0043933916000684

Salary,j.; Hemati Matin, HR.; Ghafari, K. and Hajati, H.(2017). Effect of in ovo injection of calcium carbonate nanoparticles on bone post hatched characteristics and broiler chicken performance.Iranian Journal of Applied Animal Science 7 (4), 663-667, 2017

Saleh, A. A. and Ebeid, T. A.(2019).The impact of phytoestrogen source supplementation on reproductive performance, plasma profile, yolk fatty acids and antioxidative status in aged laying hens. Reproduction in domestic animals 54 (6), 846-854, 2019

Sawosz, F.; Pineda, L.; Hotowy, A.; Sawosz, P. E.; Szmidt, M.; Niemiec, T. and Chwalibog,A. (2012). Nano-nutrition of chicken embryos. The effect of silver nanoparticles and glutamine on molecular responses, and the morphology of pectoral muscle. *B. J. C. C. S. B., 2*: 29–45.

Selim, Sh. A.; Gaafar, K. M. and El-ballal, S. S. (2012). Influence of in *ovo*administration with vitamin E and ascorbic acid on the performance of Muscovy ducks. Emir. J. Food Agric., 24 (3): 264-271.

Siekmann, B., Westesen, K. (1996). Investigations on solid lipid nanoparticles prepared by precipitation in o/w emulsions. Eur J Pharm Biopharm 43, 104-109.

Simon, J. and B. Leclercq (1982). Longitudinal study of adiposity in chickens selected for high or low abdominal fat content. Further evidence of a glucose-insulin imbalance in the fat line. J. Nutr. 112: 1961–1973

Singh, H.; S. Sodhi and R. Kaur, (2006). Effects of dietary supplements of selenium, vitamin E or combinations of the two on antibody responses of broilers. Brit. Poult. Sci., 47: 714-719

Sjostrom B, Bergenståhl B. (1992). Preparation of submicron drug particles in lecithin stabilized o/w emulsions I. Model studies of the precipitation of cholesterylacetate. Int J Pharm., 88, 53–62.

Taha, Zainab. K.; Sumaiya, N. Hawar. and Ghassan, M. Sulaiman.(2019). Extracellular biosynthesis of silver nanoparticles from Penicillium italicum and its antioxidant, antimicrobial and cytotoxicity activities.Biotechnology letters 41, 899-914, 2019.

Tako, E.; Ferket, P. R. and Uni, Z. (2004). Effects of in ovo feeding ofcarbohydrates and β-hydroxy-β-methylbutyrate on the developmentof chicken intestine. Poult. Sci., 83: 2023–2028.

Tona, K.; O. Onagbesan; Y. Jego; B. Kamers; E. Decuypere and V. Bruggeman (2004). Comparison of embryo physiological parameters during incubation, chick quality, and growth performance of three lines of broiler breeders differing in genetic composition and growth rate. Poult. Sci., 83: 507-513

Uni, Z and Ferket, R.P.(2004). Methods for early nutrition and their potential. World's Poultry Science Journal;60:101-111.

Upton, R. J.; F. W. Edens and P. R. Ferket (2008). Selenium yeast effect on broiler performance. Int. J. Poult. Sci., 7(8): 798-805.

Wahle, K.W.; Braissant, O. and Desvergne, B. (1995). Peroxisome proliferator activated receptors: Transcriptional regulators of adipogenesis, lipid metabolism and more. Chem. Biol., 2, 261–266.

Wahle, K.W.; Rotondo, D. and Heys, S.D. (2003). Polyunsaturated fatty acids and gene expression in

mammalian systems. Proc. Nutr. Soc., 62, 349–360.

Yessoufou, A.; ATEGBO, J.M.; ATTAKPA, E.; HICHAMI, A.; MOUTAIROU K.; DRAMANE KL and KHAN N. (2009). Peroxisome proliferatoractivated receptor-alpha modulates insulin gene transcription factors and inflammation in adipose tissues in mice. Mol Cell Biochem 323: 101-111.

Yoon, I.; T. M. Werner and J. M. Butler (2007). Effect of source and concentration of selenium on growth performance and selenium retention in broiler chickens. Poult. Sci., 86: 727-730.

Yoon, M. (2009). The role of PPARalpha in lipid metabolism and obesity: focusing on the effects of estrogen on PPARalpha actions. Pharmacol Res 60: 151-159.

Yuan, D.; Zhan, X.A.; Wang, Y.X.(2011). Effects of selenium sources and levels on eproductive performance and selenium retention in broiler breeder, egg, developing embryo, and 1-day-old chick. Biol Trace Elem Res. 2011;144:705–714.

Zhang, J. S.; Gao, X. Y.; Zhang, L. D. and Bao, Y. P. (2001). Biological effects of a nano red elemental selenium. Biofactors, 15(1): 27–38.

الملخص العربي

تأثير حقن أجنة البيض بجزيئات السيلينيوم النانومترية والزيت العطرى علي التعبير الجينى وعلاقته بالاستجابات الفسيولوجية لكتاكيت اللحم

أسماء الباحثين محمد سعد ابراهيم حماده1,محمد حسن عبد العزيز موسي2,ايمان احمد السعيد3,نورهان محمد شرشيرة1

1-قسم البيوتكنولوجيا الزراعية ,كلية الزراعة,جامعة دمياط

2- قسم الوراثة,كلية الزراعة,جلمعة المنصورة

3- قسم الانتاج الحيواني والداجنى والسمكي,كلية الزراعة,جامعة المنصورة

الهدف من الدراسة: دراسة تأثير حقن اجنة بيض الدجاج باستخدام جزيئات السيلينيوم النانومترية وزيت القرنفل العطري خلال فترة التفريخ وتأثيرها على تعزيز الاداء الانتاجي ومعدلات النمو للكتاكيت الفاقسة وكذلك أنماط التعبير عن الجينات المرتبطة بالنمو وتكوين اللبيبدات (IGF-1 PPARα, PPARγ) في أنسجة الكبد. تم استخدام ٦٠٠ بيضة مخصبة من قطيع امهات كتاكيت اللحم وتم تقسيم جميع البيض إلى عشر معاملات في تجربة عاملية (٢*٥) والتي تشمل السلالتين Avian ,Ross308 وتم تقسيم كل مجموعة رئيسية إلى خمس معاملات متساوية في حقن البويضات المجموعة الأولى (B1: بدون حقن)، المجموعة الثانية (B2: تم حقنها بـ ٠,١ مل من محلول ملحي، بينما تم حقن المجموعة الثالثة (B3)، الرابعة (B4) والخامسة (B5) بـ ٠,١ مل من جزيئات النانو سلينيوم وزيت القرنفل وخليط من النانوسلينيوم وزيت القرنفل علي التوالى. تم تقسيم الكتاكيت المفقسة من كل مجموعة إلى خمس مكررات وتم رفعها حتى عمر ٣٥ يومًا. وتم قياس التعبير الجيني للجينات المستهدفة من خلال انسجة خلايا الكبد بعد الدبح

وأظهرت النتائج وجود فروق معنوية بين المعاملات في كلا من وزن الكتاكيت عند عمر يوم وأداء النمو والتاثير الايجابي علي نسبة البروتين الكلي والالبيومين والجلوبيولين وخفض نسبة الكوليسترول في الدم . بالإضافة إلى ذلك، كان لـ SeNPs وزيت القرنفل تأثير إيجابي على ترسيب الدهون والتغيرات في التعبير الجيني على الدهون في دجاج اللحم. تم تسجيل اختلافات كبيرة في التعبير الجيني لجينات IGF-1 وPPARα وPPARγ بين جميع دجاج التجربة. زيادة نشاط IGF-1 وأكسدة الأحماض الدهنية بمعاملات ال SeNPs وزيت القرنفل وخليط النانوسلينيوم والزيت العطري، كذلك تعزيز نشاط IGF-1 (عامل نمو الأنسولين) باستخدام مواد الحقن. بينما أظهر جين PPARα وPPARγ انخفاضًا في نشاط النسخ في الدجاج المعامل بـ SeNPs وزيت القرنفل وخليط النانو سلينيوم والزيت العطرى معا.

لذا نستنتج من الدراسة ان يمكن حقن بيض دجاج كتاكيت اللحم الدجاج باستخدام جزيئات السيلينيوم النانومترية وزيت القرنفل العطري دون حدوث تأثيرات سلبية على الأداء الإنتاجى و معايير الدم أو التعبير الجينى للجينات ذات الصلةحيث يمكن من خلال المعاملات رفع نشاط النمو واكسدة الدهون في جسم الدجاج وكذلك خفض نسبة اللبيدات الضارة .