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Toxicology

Response of Broiler Chicken to in Ovo Administration of Nano Encapsulated Thyme Oil

Asmaa yaseen, Khaled Gaafar and Reham Abou-elkhaire

Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, University of Sadat city

**Corresponding author:* asmaaabdelrasool165@yahoo.com *Received: 10/3/2022 Accepted: 2/4/2022*

ABSTRACT:

Trail was conducted to study the effect of in ovo administration of nano encapsulated and dietary inclusion of thyme oil on broiler growth performance and carcass traits. Egg injected at 18 day of incubation with 0.03ml/egg with nano thyme oil (T3) other group injected with normalsterilesaline as positive control group (T2) and negative control group with not injected (T1). After hatching the chicks allotted to feeding trial with 80mg/kg of feed with Nano encapsulated thyme oil to study their effect on growth performance and carcass traits in relation to control non treated groups. The result showed that there was a significant($P < 0.05$) increase in body weight and body weight gain, significant ($P < 0.05$) improvement in feed consumption and feed conversion ratio, while no significant effect on carcass traits in in ovo injected and dietary Nano thyme oil relative to control group.

Key words: Broiler growth performance, Carcass traits Nano encapsulation and Thyme oil.

INTRODUCTION:

Nowadays, injecting stimulant solutions into fertilized eggs has been used to increase the reproductive and functional parameters of birds. Egg injection technology is a practical technique of injecting some of the nutrients needed by the developing embryo as well as a way to vaccinate birds against certain diseases (Foye et al., 2005 and Uni et al., 2005). Egg nutrients have been altered in a number of experiments to optimize embryonic growth in birds.

Aromatic plants are a natural source of essential oils, which are a complex blend of volatile and non-volatile plant secondary metabolites (Bakkali et al., 2008). They were used in poultry diets to stimulate or promote a more efficient use of feed nutrients, which could lead to improved weight gain and feed conversion efficiency. Herbal active ingredients may also help broilers digest their food and boost their immune systems (Ghazalah and Ali, 2008). Thyme (Thymus vulgaris), a Lamiaceae flowering plant, is a prominent medicinal plant all over the world.Thyme's active ingredients (phenolic monoterpenes thymol and carvacrol) have been shown to have antioxidant and antibacterial activities (Ouwehand et al., 2010). In the presence of oxygen from the air, EOs undergoing unwanted deterioration reactions with associated off-flavours and odours, negatively affecting the physical stability or integrity of the food chemistry as well as the loss of the biological activity of bioactive compounds (McClements, 1999).Allergens and/or products with lower biological activity than the original chemicals may result from oxidation processes (Neumann and Garcia, 1982). However, using large concentrations of EOs directly in diets affects their sensory qualities and may reduce feed consumption due to poorer palatability (Cross et al., 2003). This has necessitated the encapsulation of EOs in a robust matrix capable of overcoming the feed's technical challenges of stability and smell (Bustos et al., 2003). Encapsulation also helps to

prevent degradative processes that lead to a loss of EO quality and promotes the aggregation of incompatible ingredients in a formulation. This can result in improved feed flavour, easier handling, less dustiness, increased stability, delayed EO release in the digestive tract, and increased bioavailability (Hosseini et al., 2013). Therefore, the aim of this research is studying the effect of in ovo and dietary inclusion of Nano-encapsulated thyme oil on growth performance and carcass traits of broiler chickens.

MATERIALS AND METHODS:

Preparation of encapsulated Eos:

Encapsulation of the essential oil in chitosan nanoparticlesmade by Ionic gelation processas revealed by Stoica et al. (2013).

In ovo injection of Nano encapsulated thyme oil:

Eggs of Ross broiler strain (500) were obtained and stored in a cool-humid storage area with a temperature of 17°C and 75% relative humidity. Warming up to room temperature before placing them in the incubator. Eggs were fumigated before setting them into the incubator. On the day 18th of incubation, eggs showing viable embryo after candling them were injected with 0.03ml/egg of thyme oil as shown in table (1).

Table (1): experimental design:

Groups		Treatments
T1	Control group	The hatched chicks from non-injected eggs
T2	Nano thyme oil (fed group)	The in ovo injected hatched chicks with 0.03ml/egg with Sterile saline was fed on 80mg/kg NanoThyme oil
T3	Nano thyme group (injected and fed)	The in ovo injected hatched chicks 0.03ml/egg With Nano thyme oil were fed on 80 mg/kg Nano thyme oil

Birds and diet:

Post-hatch chicks were allocated into 3treatments and 5 replicates of 10 chicks per replicate, and reared in floor cages wood shaving for bedding. Feeders and waterers were provided based on standard management condition. The experiment lasted for 42 days, during the experiment feed and water were provided adlibitum. All diets were prepared approximately one week before the beginning of the experiment. The dietary formulation was based on the recommendation of Rose 500catalog which according to NRC (1994). All birds were fed mash feeds adlibitum throughout the duration of the experiment. All feeds were weighed and recorded prior to distribution to each pen. Standard broiler starter (0-21 d), and finisher (22-42 d) diets were provided (Table2). All of the birds had a constant access to drinking water.

Table (2): Composition and calculated chemical analyses of experiment:

Ingredients	Starter grower(0-21day)	Finisher(22-42day)
Corn	55	63.6
Soybean (44%)	35	27
Dical. P	4 3.15	
Sunflower oil	4	4.35
Common salt	1	1
Vit.Mineral.Premix*	0.50	0.50
DL.Methionine	0.35	0.25
L.Lysine	0.15	0.15
Total	100.00	100.00
Calculated Nutrient Composition		
ME (kcal/kg)	2920	3100
Crude protein (%)	19.817	
Calcium (%)	1.04	1.17
Methionine (%)	0.59	0.56
Lysine (%)	1.30	1.14
Available p. (%)	0.67	0.7

Growth performance:

a) Body weight and body weight gain:

The chicks of each group were individually weighed weekly till the end of experiment, at 1st, 7th, 14th, 21st, 28th, 35th, and 42nd days of age. The differences in the body weight were calculated and recorded. The body weight gain was calculated for each group from the difference between the weight at the beginning and at the end of 1st, 2nd, 3rd, 4th, 5th and 6th weeks of age according to Brady(1968) using the following formula:

Body weight gain = W2 – W1

Where:

W1: body weight at the beginning of experiment.

W2: body weight at the end of the period.

b) Feed consumption and conversion rates:

A known weighed amount of ration was putted to birds of each group daily in the morning then at the end of each week the remaining amount was weighed and subtracted from the original amount of ration. The average amount of feed consumed per chick was calculated after 1st, 2nd, 3rd, 4th, 5th, and 6th weeks of feeding. Feed conversion ratio (FCR) was calculated according to Brady (1968) using the following formula:

$$FCR = \frac{\textbf{Feed consumption in a given period}}{\textbf{Gain produced in the same period}}$$

Carcass traits:

For carcass evaluations, 10 birds per treatment were selected based on the average weight of the experimental unit. The carcasses were manually eviscerated and breast, legs, back, gizzard, liver, heart, abdominal fat and lymphoid organs (spleen, bursa of Fabricius and thymus). The carcass yields were calculated as a percentage of the live weight and the other parts yield was expressed as the percentage of carcass.

Statistical analysis:

A completely randomized design was used. Measurable examination for the acquired information was performed by one-way analysis of variance (SAS, 2006). Duncan's new multiple range test was utilized to separate significant differences among means (Duncan, 1955).

RESULTS:

Table (3): The effect of Nano encapsulated thyme on broiler body weight*mean± SE:

	T1	**T2**	**T3**
Initial body weight at day 0	48.14±0.122^{c}	49.23±0.202^{b}	52.267±0.202^{a}
BWt at week1	159.8±1.62^{b}	203.6±3.1^{a}	196.6±10.31^{a}
BWt at week2	418.9±9.18^{c}	456±12.35^{b}	493.4±7.97^{a}
BWt at week3 (21 day)	773.7±11.51^{c}	859±15.62^{b}	966.9±14.4^{a}
BWt at week4	1418.8±8.32^{c}	1455.7±14.27^{b}	1598.4±14.7^{a}
BWt at week5	1812.6±14.74^{c}	1862.3±19.13^{b}	2000.2±31.85^{a}
BWt at week 6 (42 day)	2224.1±15.39^{c}	2427.9±21.13^{b}	2635.3±39.85^{a}

a_ b_ c _d means within the same rows having different superscripts are significantly different ($p < .05$). **BWt**: weekly body weight of bird.

T1: (Control group) The hatched chicks from non-injected eggs**, T2**:Nano thyme oil (fed group) The in ovo injected hatched chicks with Sterile saline were fed on 80mg /kg Nano thymeoil**, T3:**Nano thyme oil (in ovo injected and fed group): The in ovo injected hatched chicks with Nano thyme oil were fed on100mg/kg Nano thyme oil.

Table (4): The effect of in ovo injected and fed Nano encapsulated thymeon broiler weight gain (Wt gain) *mean± SE:

	T1	**T2**	**T3**
Wt gain from day0 to 7 day (Wt gain 1)	110.8±1.62^{b}	155.6±2.87^{a}	157.3±4.71^{a}
Wt gain from 7 to 14 day(Wt gain 2)	258.9±7.93^{b}	257.7±8.61^{b}	157.3±4.71^{a}
Wt gain from 14 to 21 day (Wt gain 3)	352.5±4.85^{c}	405.1±4.90^{b}	464.5±8.10^{a}
Wt gain from 21 to 30 day (Wt gain 4)	642.2±4.03^{a}	597.2±2.7^{b}	638±5.14^{a}

Wt gain from 30 to 37 day(Wt gain 5)	398.5±7.38^{c}	401.4±6.81^{b}	610.6±19.03^{a}
Wt gain from 37 to 42 day(Wt gain 6)	411.3±4.35^{b}	564.3±7.74^{a}	423.5±11.36^{b}
Total Wt gain (0-42 day)	2174.4±15.3^{c}	2381.5±20.0^{b}	2587.0±42.14^{a}

a_ b_ c _d means within the same rows having different superscripts are significantly different ($p < .05$). BWG: weekly body weight gainof bird.

T1:(Control group) The hatched chicks from non-injected eggs, T2:Nano thyme oil (fed group) The in ovo injected hatched chicks with Sterile saline were fed on 80mg /kg Nano thyme oil, T3:Nano thyme oil (in ovo injected and fed group): The in ovo injected hatched chicks with Nano thyme oil were fed on100mg/kg Nano thyme oil.

Table (5): The effect of in ovo injected and fed Nano encapsulated thymeon feed conversion ratio of broiler chicken performance *mean± SE:

	T1	**T2**	**T3**
FC from day 0 to 7 day (Fc 1)	1.27± 0.018^{a}	0.99± 0.025^{b}	0.97± 0.029^{b}
FC from 7 to 14 day (FC 2)	1.29± 0.026^{a}	1.24±0.037^{b}	1.11± 0.017^{c}
FC from 14 to 21 day(FC 3)	1.46± 0.021^{a}	1.32± 0.015^{c}	1.44± 0.021^{b}
FC from 21 to 30 day (FC 4)	1.52±0.024^{a}	1.29±0.008^{c}	1.39±0.011^{b}
FC from 30 to 37 day (FC 5)	1.82±0.034^{a}	1.73±0.028^{a}	1.53±0.050^{b}
FC from 37 to 42 day (FC 6)	2.31±0.025^{a}	1.83±0.035^{b}	1.86±0.048^{c}
Total FC (0-42 day)	2.11±0.015^{a}	1.86±0.016^{b}	1.65±0.027^{c}

a_ b_ c _d means within the same rows having different superscripts are significantly different ($p < .05$). FCR: feed conversion ratio of bird.

T1: (Control group) The hatched chicks from non-injected eggs, T2:Nano thyme oil (fed group) The in ovo injected hatched chicks with Sterile saline were fed on 80mg /kg Nano thyme oil, T3:Nano thyme oil (in ovo injected and fed group): The in ovo injected hatched chicks with Nano thyme oil were fed on100mg/kg Nano thyme oil.

Table (6): The effect of in ovo injected Nano encapsulated thymeon broiler feed intake *mean± SE:

	T1	**T2**	**T3**
FI from day0 to day 7 (FI 1)	140.71±0.96^{b}	141.53±0.94^{a}	138.80±0.63^{b}
FI from 7 to 14 day (FI 2)	326.34±1.06^{a}	319.32±0.97^{b}	324.12±0.83^{a}
FI from 14 to 21 day (FI 3)	592.69±0.96^{a}	589.62±0.92^{a}	511.62±0.90^{b}
FI from 21 to 30 day (FI 4)	932.46±1.003^{a}	907.37±0.707^{b}	883.36±1.35^{c}
FI from 30 to 37 day (FI 5)	1179.1±2.59^{a}	1138.1±1.38^{b}	1107.7±2.011^{c}
FI from 37 to 42 day (FI 6)	1432.0±1.84^{a}	1351.6±2.25^{b}	1287.2±2.13^{c}
Total FI (0-42 day)	4603.3±2.64^{a}	4461.9±2.68^{b}	4252.8±3.80^{c}

a_ b_ c _d means within the same rows having different superscripts are significantly different ($p < .05$). FI: weekly feed intake/bird.

T1:(Control group) The hatched chicks from non-injected eggs, T2:Nano thyme oil (fed group) The in ovo injected hatched chicks with Sterile saline were fed on 80mg /kg Nano thyme oil, T3:Nano thyme oil (in ovo injected and fed group): The in ovo injected hatched chicks with Nano thyme oil were fed on100mg/kg Nano thyme oil.

Table (7): The effect of in ova injected and fed Nano encapsulated thymeon broiler carcass traits*mean± SE:

	T1	**T2**	**T3**
LBW	2204.2±45.39^{a}	2331.7±72.18^{a}	2326.7±76.75^{a}
DW	76.56±1.47^{a}	77.12±1.19^{a}	77.26±1.15^{ab}
Breast M	20.03±1.09^{b}	22.02±1.37^{ab}	24.81±0.79^{ab}
Thigh M	15.03±0.38^{a}	15.24±0.85^{a}	14.93±1.02^{a}
Drum stick M	5.41±0.40^{a}	5.22±0.28^{a}	5.76±0.39^{a}

Abdominal fat	0.94±0.19^{b}	1.46±0.12^{ab}	1.39±0.08^{ab}
Liver	2.05±0.13^{a}	1.83±0.16^{a}	1.66±0.07^{a}
Gizzard	1.51±0.10^{a}	1.75±0.13^{a}	1.58±0.12^{a}
Heart	0.34±0.02^{a}	0.36±0.018^{a}	0.32±0.01^{a}
Spleen	0.08±0.005^{a}	0.05±0.005^{b}	0.075±0.005^{ab}
Bursae	0.049±0.003^{a}	0.037±0.0024^{b}	0.045±0.0054^{ab}
Thymus	0.143±0.010^{a}	0.084±0.011^{c}	0.116±0.007^{b}

a_ b_ c _d means within the same rows having different superscripts are significantly different ($p< .05$). LBW: live body weight of bird. DW: dressing weight of bird

T1:(Control group) The hatched chicks from non-injected eggs, T2:Nano thyme oil (fed group) The in ovo injected hatched chicks with Sterile saline were fed on 80mg /kg Nano thyme oil, T3:Nano thyme oil (in ovo injected and fed group): The in ovo injected hatched chicks with Nano thyme oil were fed on100mg/kg Nano thyme oil.

DISCUSSION:

Growth performance:

As shown in table (3), (4),(5),(6) there was a significant ($P< 0.05$) increase in body weight (BWt) and body weight gain in groups treated with Nano essential oils in all weeks' relative control group(T1). from the first week till the sixth week the highest body weight was recorded in ovo injected and fed Nano thyme oil (T3) followed by groups fed on Nano thyme oil (T2) relative to control one. Moreover, there were a significant ($P< 0.05$) improvement in feed intake and feed conversion ratio in in ovo injection and fed nano thyme oil (T3) followed by (T2) relative to control all over rearing period. The results were in agreement with the finding of Toosi et al. (2016) who found that in ovo injection of Biacid^{TM} improved final body weight gain, FCR in the treatments ($p<0.05$) of *in Ovo* Injection at day 18th of incubation with a Blend of Essential Oils and Organic Acids (Biacid^{TM}) in High NSPs Diets of Broiler Breeders .Moreover, Sulaiman and Tayeb, (2021) studied the Effect of in-ovo injection of different doses of rosemary oil on broiler growth performance and they revealed that At day 7, At 21 days and At35 days of age of broiler the highest body weight and the bodyweight gain of birds was recorded in eggs groups injected with rosemary oil were increased compared to control group from (0.1, and 0.075 ml followed by 0.05 ml of rosemary oil injected eggs compared to control groups. While, FCR was significantly ($P<0.05$) better in group injected with Rosemary oil (0.05ml) and (0.1ml) than (NC), PC (injected with distilled water 0.05ml) and (0.075 ml rosemary oil).

There is a scarcity of information on the in ovo injection of Nano encapsulated essential oil however, dietary inclusion of Nano encapsulated essential oils improved body weight of birds, as revealed by Hosseini and Meimandipour (2018) who studied the effect of thyme oil encapsulated with chitosan on broiler performance which increasing BWG and FCR at different growing periods. This confirmed by Nouri, (2019) who studied the effects of chitosan Nano-encapsulating mint (MEO),thyme (TEO) and cinnamon (CEO) essential oils (EOs) used in the diet on broiler growth performance and revealed that both essential oils and the chitosan Nano encapsulated form had significant ($P < 0.05$) benefits on improving body weight gain and FCR from 1–42 days. The better effect was reported in birds with Nano encapsulated TEO. Moreover, Encapsulation of EOs increased the BWG compared with the control group as reported by Heydarian et al. (2020) who studied the effect of encapsulated and non-capsulated thyme and oregano essential oils on growth performance and carcass traits, they found Encapsulation of the EOs could increase BWG and FCR in comparison with non-capsulated form on days 24 and42; suggesting that encapsulation can efficiently increase BWG. The improvement in BWG and FCR of the broilers fed with the TEO could be due to stimulation of digestive system and immune system by the active compounds (especially carvacrol, linalool and thymol) in thyme oil (Abdulkarimi et al., 2011; Petrolli et al., 2012; Ramadan, 2013). Thymol and carvacrol are the majorcomponents of TEO, which have a better effect on growth performance in broilers (Allen et al., 1998; Denli et al., 2004).

Carcass traits:

The data of carcass parameters are shown in Table (7) It could be concluded that the treated groups in ovo injected and fed non thyme oil groups (T3) and fed groups (T2) did not have any significant effect on weight of (heart, gizzard, liver). while, Percentages of immunity organs (spleen, bursae, thymus) as indicators of immune situation were improved in the treated groups than control ones. Moreover, there was a significant ($P< 0.05$) increase in weight of dressing weight, breast muscle and abdominal fat of broiler chicken in treated groups than control ones. The result was in agreement with Toosi et al. (2016) who reported that the in Ovo Injection at day 18th of incubation with a Blend of Essential Oils and Organic Acids (Biacid^{TM}) in High NSPs Diets of Broiler Breeders did not have significant effects on carcass yield. Also the abdominal fat had not affected by in ovo injection of Biacid^{TM}. Moreover, Nouri, (2019) revealed that chitosan nano-encapsulating mint (MEO),thyme (TEO) and cinnamon (CEO) essential oils (EOs) had no significant ($P > 0.05$) effect on carcass traits. While, the effect of CNE form was only significant ($P < 0.05$) for increasing breast meat of broilers. Furthermore, the effect of encapsulated and non-capsulated thyme and oregano essential oils on growth carcass traits reported by (Heydarian et al., 2020) who found that dietary inclusion of Eos (encapsulated and non-capsulated) and probiotics did not have significant effects on carcass traits except higher gizzard weight with The birds fed diets containing antibiotics, and probiotic plus encapsulated TEO+OEO.

CONCLUSION:

It was concluded that the in ovo injection and dietary inclusion of Nano encapsulated of thyme oil improve broiler chicken growth performance and conversion ratio with little effect on carcass traits in relation to control non treated group.

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