Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Influence of Thyme Oil Nano-Encapsulated Chitosan on Productivity, Growth Performance, and Meat Quality of Japanese Quails

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Abstract

THIS research examined how productive, physiological, and meat quality characteristics are affected by thyme oil Nano chitosan-Encapsulated (ETEO).The control group (T1) consumed the basal diet without supplementation, while the second and third groups fed control with Thyme essential oil (TEO) at 50 mg/kg diet (T2) or 70 mg/kg diet (T3). Finally, the fourth and fifth groups fed control +ETEO at 30 mg/kg (T4) and 50 mg/kg (T5) until 42 days of age. Compared to the control group, quails given a (T4) diet had the greatest body weight (BW), feed intake (FI) at 28 days, crude protein (CP) intake, and metabolizable energy (ME) intake from 7 to 42 days old. In terms of meat quality, the T4 and T5 groups had considerably lower TBARS levels ($P<0.05$) than the control and other groups. Control quail meat (thigh and breast) had the lowest total volatile basic nitrogen (TVB-N) ($P<0.05$), whereas T4 had the lowest quantity. The supplements improved blood total antioxidant capacity (TAC) in birds fed (T3) relative to (T2) and the control group. Both T2 and T3 showed considerably decreased circulating low-density lipoprotein (LDL) ($P<0.05$). T4 and T5 feeding increased TP and G levels considerably. As conclusion: Chitosan Nano-Encapsulation could be a method for retaining compounds, properties, and effects of the essential oils used in low concentrations and improving the meat quality of quails.

Keywords: quail, growth, physiological characters, meat quality, Thyme oil, nano-capsulated, chitosan.

Introduction

The global trend to replace the use of antibiotics as growth promoters with safe feed additives in the industry has led researchers to conduct a massive exploration into utilizing natural substance-based additives. Essential oils (EO), have volatile properties derived from plant materials by mainly steam distillation method [1]. The advantageous effects of EO are associated with their role on many metabolic pathways, including lipid metabolism, stimulating digestive enzyme secretion and activity, acting as an antimicrobial, and enhancing gut integrity of chickens [2] leading to improve broiler performance in general. However, inconsistent results among past and present studies are identified to be conflicting. For instance, there were positive effects of EO on broiler performance as indicated by improving BWG [3-4], FCR [5], enzyme secretion [6] and nutrient digestibility [7] Herbal essential oils can be used as an antibiotic to improve poultry performance, especially in intensive management systems [8]. Thyme (Thymus vulgaris) is commonly used as a spice in human food and has received considerable attention as a beneficial additive in poultry nutrition.

The positive effect on poultry health and performance may be achieved through the stimulation of appetite and feed intake, improvement of endogenous enzyme secretion, activation of

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(Received 17/12/2023, accepted 09/02/2024)
DOI: 10.21608/EJVS.2024.255678.1730

immune responses, and antioxidants [9]. However, not all researchers have found any improvement in the performance of broilers. Carcass traits have similarly been found by some researchers to be unresponsive to thyme oils [10] Thyme essential oil (TEO) can also stimulate the immune system by increasing the activity of lymphocytes, macrophages, and NK cells, as well as increasing phagocytosis and interferon synthesis [11]. TEO bioactive chemicals' high activity, hydrophobia, reactivity, volatility, and peroxidation sensitivity restricts their usage in poultry feeds. Thus, such unfavorable reactions may reduce oil efficiency, natural flavors, and sensory quality, lowering palatability and FI, particularly in diets rich in TEO [12]. To increase the delivery and controlled release of active substances, microencapsulation or Nano-Encapsulation technology may entrap them in the core of the membrane wall structure of Nano-capsules or adsorb them onto a carrier [8]. Polyphenol degradation and sluggish gastrointestinal release may be reduced by encapsulating plant extracts in nanoparticles [13].

Deacetylated chitin, chitosan, is widely used. Chitosan is found in prawns, crabs, krill, and other crustacean exoskeletons and seems to improve animal performance [14]. Chitosan also improves immunity and has antibacterial properties [15]. Chitosan, a linear polysaccharide made by alkaline deacetylation of chitin, is employed as a matrix in pharmaceuticals and EO owing to its non-toxicity, film-forming capability, permeability, high much-adhesivity, and high tensile strength [16]. Due to the interplay of charges between its polycationic amino groups and bacteria cell walls, chitosan is a potent antimicrobial [17]. Chitosan also improves broiler chicken development [14] and plasma biochemical markers [18]. Its antioxidant and antimicrobial activities increase ileal digestibility [19]. Nano-chitosan, a natural biopolymer made from chitin, has received interest in agriculture owing to its antibacterial, immunological modulating, and antioxidant capabilities. Encapsulating nano-chitosan improves its stability, bioavailability, and prolonged release, enhancing its medicinal potential [20]. Additionally, dietary chitosan and Nano-chitosan increased quail performance, carcass features, antioxidant status, and plasma constituents [21].

The current research examined the effects of nano-encapsulating thyme in-feed on quail productivity and immunological responses. FI was higher in ETEO groups than in TEO alone.

Material and Methods

Experimental designs

The experiments were done at El-Fayoum Poultry Farm, Animal Production Research Institute, Agriculture Research Centre, Ministry of Agriculture, Egypt. Management All international, national, and institutional, 400 unsexed Japanese quail-7-day-old birds with almost similar live weights (49g) were randomly assigned into five treatment groups. Each treatment group included 80 birds, four duplicates, and a 20-bird replicate. The birds were housed in a conventional type cage (50 × 30 × 50 cm3).

Birds were fed the basal diet (Control, T1), treatment groups were thyme essential oil with two different concentrations at 50 mg/kg (T2), 70 mg/kg (T3), thyme oil capsulated Nano-chitosan with two different concentrations at 30 mg/kg (T4), and 50 mg/kg (T5).

Throughout the study, birds were fed and watered ad libitum in 24h light. ABWG, FCR, BW, FI, and MR were recorded weekly. After 42 days, three birds from each treatment were killed to acquire the carcass and weigh the giblets (Gizzard, liver, and heart) and lymphoid organs.

Birds ate 2900 Kcal and 24% CP iso-nitrogenous food. The baseline diet was designed for Japanese quail (JQ) needs by NRC [22]. The basic diet composition and calculation are shown in Table 1.

Capsulated thyme oil with nano-chitosan preparation

Thyme oil was kindly purchased from the oils extract unit of the National Research Center (NRC), Tween 80 was obtained from the Sigma-Aldrich Co., deionized water and chitosan obtained from the Sigma-Aldrich Co. Preparation of thyme oil micro-emulsion (3% and 5% oil in water) was done in nanomaterials Research and synthesis unit by using the method according to [23].

Capsulated thyme oil with nano-chitosan characterization

Zetasizer Malvern Instrument (Corp, Malvern, UK) was used to measure droplet size, surface charge (zeta potential), size distribution (PDI), and electrical conductivity of encapsulated thyme essential oil with nano-chitosan (ETEO). A JEM 1400F HRTEM at 300 keV conducted high-resolution transmission electron microscopy (HRTEM) studies. Central laboratory, Faculty of Agriculture, Cairo University, GC-MS thyme oil and micro-emulsion components.

Cell culture: Vero: Green monkey cell line from Nawah Scientific Inc. (Mokatam, Cairo, Egypt). In humidified, 5% (v/v) CO_2 at 37 °C, cells were maintained in DMEM medium with 100 mg/mL streptomycin, 100 units/mL penicillin, and 10% heat-inactivated fetal bovine serum. Cytotoxicity test: SRB (sulforhodamine B) assay with doses of 0.01, 0.1, 1, 10, and 100 ug/mL measured cell viability [24].

Gross Chemical composition and meat quality

Moisture content, protein, Fat content, and Ash content According to the method described by the A.O.A.C [25].

Meat quality tests: Six samples per treatment/test of breast and thigh muscle were stored frozen at -20 °C for 60 days for determination.

Thiobarbituric acid-reactive compounds.

One of the most reliable techniques for quantifying malondialdehyde (MDA) in animal tissues is TBARS. Since it measures MDA concentration, TBARS is a good oxidation monitor. Tarladgis *et al.* [26] tested muscle tissues for TBARS. TBARS levels were measured in MDA micrograms per gram of tissue.

Water Holding Capacity (WHC) and Plasticity

Water retaining capacity and plasticity were tested using [27]. This procedure included pressing 0.3 g of quail (breast and thigh) flesh tissues beneath ashless filter paper (Whatman No. 41) for 10 minutes with a kg weight. Meat samples were prepared on filter paper and measured 46 surface areas using a planimeter. The outer zone from water separated from pressed tissues indicated the WHC index in Cm2. Each I Cm2 outer zone area equals 8.4 mg free water. The bound water % of moisture content was estimated as follows.

To calculate the bound water percentage, divide moisture content by 8.4 × cm^2 outer zonarea (moisture content) by 1000 × 0.3.

Cooking loss

A 1 cm^3 slice of meat from each muscle sample was weighed to evaluate cooking loss. After weighing (weight 1), the sample was maintained at 4 °C for 24 hours before being cooked in an 85 °C water bath for 10 minutes to reach 75 °C. After gently dabbing, the meat sample was reweighed (weight 2) [28]. Cooking loss was calculated using this equation:

Cooking loss = 100 - (Weight 1 - Weight 2)/Weight1

2.7. Biochemical blood analysis: Haematological parameters, blood concentration of total protein, glucose, albumin, globulin, total cholesterol, liver enzymatic activity (AST and ALT), uric acid, creatinine, MDA, and SOD were measured using Biodiagnostic Company kits.

Statistical analysis

Data were analysed using xlstat software, a general linear model, for one-way analysis of variance [29].

This model was used: $Yij = \mu + Li + eij$

Where: Yij: The jth observation in the ith line, : The overall mean, Li: Treatment effect, and Eij: Random error.Data are presented as LSM ± standard errors (SE). Duncan's multiple range test separated significant mean values [30]. The significance threshold was 5%. Percentage data were arc sine transformed before analysis.

Results and Discussion

Characterization of Capsulated thyme oil with nanochitosan:

Nano-capsulated characterization of the nanodroplet was mainly determined by TEM which 5% nano-capsulated size had 33.04 nm and 3% micro-emulsion size had 13.95 nm with a narrow size distribution, (polydispersity index: 0.883 and 1, respectively) which indicated that greater homogeneity can be realized (Fig 1a, b).

The zeta potential is an indicator that stable suspensions are generally taken by using dynamic light scattering (DLS) had a 28.86± 7.42mV in 3% sol. and 20. 6± 10.6 mV in 5% sol., both have the same viscosity of 0.08872 (cp) and conductivity of 0.41±3.66 ms/cm. When GC-Mass was analyzed the thymus oil had 9 components which were Thymol (24.68%), Camphor(1.35%), o-Cymene (4.98%), Geraniol(1.55%), Hexadecanoic acid (24.24%), vaccenic acid (11.54%), cis-vaccenic acid (26.07%), C10H9Cl9 (1.62%) and 1,3-Diolein (1.26%); while nano-capsulated had many components which are Thymol (12.18%), Eugenol (18.59%), Eupalyptol (4.56 %), oleic acid, 3-(octadecyl oxy) propyl ester (32.94%), Behenyl palmitoleate (27.44%), cyclopren (5.63%) and Erucyl gondoate(23.36%). thymus oil 3% nano-capsulated had many componentsCitronellol (4.19%), Menthol (3,10%), α –Guaiene (2.60%), Tribehenin (13.56%), 1,2-Dipalmitoyl-rac-glycerol (8.51%),Ayanin (8.62%), 2-Ethyldecyl 2-ethylundecyl phthalate (11.28%), α-Sitosterol (1.80%), β-Sitosterol (10.5%), Anthracene (10.98%), Tridecanoic acid (2.97%), Corynoxine and Marinosin (2.33%).

On the confluent surface of Vero cells, results for thyme 5% nano-capsulated had different concentrations (0.01, 0.1, 1, 10, 100 ug /ml) after 3 days of inoculation, the effect on cell viability % was assessed by SRB assay was 99.67%,99.43%, 93.89%,84.73%,68.43%, respectively in 100 ug/ml and IC50> 100 ug/ml. (Fig.1 c).

The fabrication of nano-emulsions with lesser droplet size in the presence of double bonds in the nonpolar chain of non–ionic surfactants was obtained

results were in agreement with different results [31]. The conductivity of the nano-emulsions increased by increasing essential oil concentration which demonstrated that water is the continuous phase ($P<0.05$). This is because the conductivity of the solutions is directly proportional to the number of ions and increases by increasing the ions. The cell viability reached 88-90% for nano-emulsions containing thyme and rosemary oil respectively which showed that prepared nano-emulsions are safe and non-toxic. Moradi and Barati, [32] and Kumari *et al.,* [33] reported that the stability study for the characterization of thymol based nano-emulsions had spherical droplets size (293±2.7nm), PDI (0.15) and zeta-potential (−32 mV) in 50 min sonicated as compared to other nano-emulsions. The results are strengthened by the fact that greater sonic energy flows to emulation through greater surfactant adsorption on hydrophobic droplet surfaces and helps to reduce the droplet dimensions and the spread of small droplets. Reduced droplet size and dispersion hinder the growth of droplets due to the inhibition of coalescence, focus, and maturation of Ostwald.

The optimized s 0.82% of thymus oil microemulsions was pale yellow to amber transparent microemulsion with a globule size of 14.23 ± 0.3 nm, a zeta potential of -0.69 mV, and a PDI value 0.00143 indicating a stable microemulsion [34]. Therefore, chitosan nano-encapsulation could be a method for retaining compounds, properties, and effects of the EOS used in low concentrations.

Growth performance

The effect of thyme essential oil alone or capsulated with nano-chitosan added to the diet on the productive performance of quail is represented in Table (2). the body weight at 7, 28, and 42 days of 5 treatments on dietary presented to quail There is no significant difference in all treatments at different ages except body weight at 28 days. (T3) treatment has a significant difference which has a higher body weight of 173 gm compared to the control group(T1) and (T2) (Table 2).

The results indicated that the effects of thyme essential oil and thyme essential oil capsulated with nano-chitosan added in the diet were significant ($P < 0.05$) for feed intake, crude protein intake, and ME intake, (T4) significantly increase feed intake, crude protein intake, and ME intake compared to the control group. Also, had a good effect on the efficiency of crude protein intake and ME intake compared to other treatments but no significant effect. However, Thyme essential oil alone or capsulated with nano-chitosan added to the diet had no significant effect ($P > 0.05$) on body weight gain, growth rate, and feed conversion ratio compared to the control.

In this study, thyme essential oil coated with nano-chitosan increased feed intake compared to thyme essential oil alone, and this indicates that nano-encapsulation technology increases the palatability of quails for herbal essential oils. However, this result does not completely correspond to an improvement in body weight gain and FCR of the broilers fed with the chitosan nano-encapsulating thyme essential oils (0.025%, 0.04%, and 0.055% respectively to starter, grower, and finisher diets) in the Nouri, [8] study. Also, Lee et al., [35] used 100 mg/kg of thymol and cinnamaldehyde in a broiler diet but did not find any significant effect on feed intake.

In addition, Hosseini and Meimandipour, [36] found that adding thyme essential oil into the diet significantly ($P<0.05$) improved FCR compared to the control group, and increased body weight gain compared to the control group but did not affect feed intake. It should be noted that the results of growth performance in broiler chickens from several studies were different, possibly owing to a variety of chitosan characteristics such as concentration, degree of acetylation, and molecular weight [37], As well as the difference in the types of birds.

Carcass characteristics were not significantly different (Table 3). However, cecum length increased in the dietary thyme essential oil capsulated with nano-chitosan treatments (T4 and T5) compared to the control group.

Meat chemical composition

The approximate chemical composition of quail meat as shown in Table (4) revealed that the lowest moisture content was 66.1% for treatment 4 and the highest percentage was 67.3±0.9% for treatment 3. As for ash, the highest percentage was (1.31±0.12%) for control, and the lowest percentage was (1.04±0.12%) for treatment 1. While the protein and fat contents were values in the range of (22.48±0.88 to 23.35±0.88 %) and (8.72±0.84 to 11.43±0.84 %), respectively.

Regarding quality parameters of quail's meat composition (thigh and breast together). As shown in Table 4, there are no significant differences between the treatments.

Meat quality traits

The findings of meat quality characteristics which was indicate that all treatments exhibited significantly lower values of TBARS, TVB-N, and cooking loss% compared to the control group, with a significance level of $P \leq 0.01$. Conversely, the WHC % values were significantly higher ($p \leq 0.01$) in the

treatments compared to the control group. Specifically, both treatments involving birds fed with thyme essential oil capsulated with nano-chitosan (T 4 and T5) exhibited significantly ($p \leq 0.01$) favourable values as shown in Table (5).

The data of the current study showed that TBARS values of treatments control(T1),2, and 3 were significantly higher than those of Thyme oil Nano capsulation (treatments 4 and 5) may be due to the antioxidant properties in thyme that protect against lipid oxidation, it is more effective which, it is in the form of a nano-capsulation.

The lack of effects of treatments on different carcass traits agrees with the results of [38, 8, 9] who found no significant effect of thyme on carcass traits. The abdominal fat weight was affected primarily by the supplements, regardless of dose, being reduced by 41% for chickens fed the thyme supplements and 62% for chickens fed the sumac supplements. This reflects reduced low-density lipoproteins in the blood.

Such a finding coincides with that obtained by Khalifa, *et al.* [39] who found that the moisture and protein contents ranged from (69.87 to 72.35%) and (21.65 to 24.20%), respectively.

These antioxidant properties are due to the presence of carvacrol and thymol, thyme can extend the shelf life and improve the quality of meat products [40]. Natural oil thyme contains phenolic components that react with lipid and hydroxyl radicals to form resistant chemicals [41]. In comparison to free Thyme essential oil, Hassani, and Hasani, [42] found that E- TEO had better antioxidant activity.

The breakdown of bacterial and enzymatic proteins, as well as non-protein nitrogen molecules in meat, produces total volatile basic nitrogen (TVB-N). TVB-N stands for trimethylamine (generated by bacterial deterioration), dimethylamine (made by autolytic enzymes during storage), and ammonia measurement, food deterioration is linked to other combinations of volatile nitrogen bases [43].

In the current study, there are significant differences between control (T1) and other treatments 2, 3, 4 and5. Table (4) revealed that the highest amount of TVB-N (mg/100 g) of quail's meat (thigh and breast together) was 13.29 (mg/100g) for control(T1), while the lowest amount was 4.86 (mg/100g) for treatment 4, the decreased amount of TVB-N in other treatments (when compared to the control group) can be related to the reduction of bacterial populations in those treatments, as well as the reduction of bacteria's oxidative ability in the separation of amines from non-volatile nitrogen compounds in of quail's meat, this may be due to the effect of Thyme essential oil encapsulation as a strong antimicrobial effect [44] .

The water holding capacity (WHC) increased significantly with the samples treated with thyme oil nano-capsulation, as evident in the treatments 3 and 4 (69.95and 70.80%, respectively) followed by treatment 1 (68.46%) and 2 (65.80%), treated with thyme oil free while the control sample (63.13%) was the least of WHC, and as it is clear from Table 4 that there are significant differences between the different treatments. Similar results were obtained by Mehdipour, *et al.* [45] who suggested that feeding quails on thyme extract led to an increase in the WHC, as it was 69.62%, compared to control 66.00%. There is a significant difference between the control and treatment 1 and 4 in the cooking loss % as shown in Table 4, where the highest cooking loss was 23.55% for control, while the lowest was 20.17% for treatment 4.

This means that feeding quails, whether on free or nano-capsulation thyme oil, had a clear effect in reducing the rate of cooking loss. These findings are in a close agreement with that presented by Mehdipour, *et al.* [45] who observed that the meat samples of quails that were fed thyme extract had a cooking loss of 22.87 % less than the control which was 23.87 %.

Blood biochemical analysis

As shown in Table 6, dietary treatments did not significantly ($P>0.05$) affect blood glucose. T2 were significantly ($P<0.05$) higher than control and other groups. Whereas, (T3) was significantly higher than control (T1) in Albumin/ globulin ratio, Adding ETEO into the bird's diet and globulin compared to the control group.

Both total plasma protein and globulin levels as compared to the control, while the values for TEO-supplemented groups were intermediate. Albumin was not affected by dietary treatments.

At Table (7), Shows that Birds fed diets containing TEO (T4 and T5) had significantly TG ($P<0.05$) compared to the control group and other groups.

As in Table (8), showed that birds fed diets containing TEO (T3) had significantly ($P<0.05$) lower ALT, ALT/AST ratio, and uric acid compared to the control group, and other groups.

While albumin concentration was not affected by dietary treatments [46]. These data clearly showed that the dietary impact of E-TEO and TEO alone on total plasma protein and albumin was not significant whereas effective delivery of TEO plus its

combination with chitosan in the form of E- TEO synergistically influenced and improved immunity in birds [36].

Other findings indicated that TEO itself enhanced plasma proteins [47]. The increase in total plasma protein in birds fed diets containing EOs might have been due to the protein synthesis stimulation effects of plant-derived compounds in relative organs [48]. Moreover, the increase in globulin fraction and immunity status illustrated the effective role of TEO in developing and protecting cells and inhibiting non-enzymatic oxidation [49]. An increase in the value of plasma total protein, albumin, and globulin levels means better animal immunity [50]. Gumus *et al.* [51] found that the groups that received thyme essential oil, were lower in low-density lipoprotein (LDL) levels.

Aghazadeh *et al.* [52] have found reductions in total cholesterol and LDL in chickens supplemented with thyme extract. We also found reduced HDL in treatments T2 and T3, in contrast to Aghazadeh *et al.* [52] who did not find any effect of thyme extract on HDL. The reduction in circulating triglycerides in T3 and T4 is almost certainly connected with reduced abdominal fat deposits and was also found by Aghazadeh *et al.* [52]. Circulating plasma lipids are responsible for nearly all of the fatty acids in adipose tissue and de novo synthesis of fatty acids is limited. The latter is enhanced by insulin and reduced by thyroxine and glucagon. circulating LDL in broiler chickens decreased (31%) on day 42 in the groups fed a diet supplemented with TEO compared with the control, thus showing that thyme can enhance lipid catabolism and reduce fat deposition. This result may be due to the presence of phenol and flavonoids in TEO that may be able to increase LDL receptors on the liver cell surface and mediate the uptake and lowering of plasma LDL [53]. Although not significant, birds fed E-TEO showed a 26% reduction in LDL in plasma compared to the control birds.

These results agreed with A study conducted by Placha *et al.* [54] examined the effects of dietary thyme oil on broiler chicken health. It was observed that thyme oil supplementation significantly reduced the activity of liver enzymes, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), indicating improved liver health and function. These results agreed with several studies have reported the following findings: Reduced Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) Levels: ALT and AST are enzymes involved in liver function. Elevated levels of these enzymes are indicative of liver damage. A study by Sariözkan *et al.* [55] showed that TEO reduced ALT and AST levels in broiler chickens, suggesting a protective effect on liver health.

Blood serum oxidant and antioxidant indices:

As shown in Table (9), dietary treatments did not significantly ($P>0.05$) affect malondialdehyde (MDA), or total superoxide dismutase (SOD). While TAC was significantly affected primarily by the supplements, being increased for birds fed the thyme essential oil at 70 mg/kg diet compared to birds fed the thyme essential oil at 50 mg/kg diet and birds in the control group.

The jejunum is the main site of lipid absorption in chickens. The reduction in abdominal fat and circulating triglycerides was likely due to the inhibition of hepatic 3-hydroxy-3-methyle glutaryl coenzyme A (HMG-CoA) reductase activity that thyme produces. This is a key regulatory enzyme in cholesterol synthesis. Thyme also has saponins, which are complex with cholesterol and prevents its absorption. In our study thyme also reduced blood glucose.

Antioxidant Effect: Toxic compounds can induce oxidative stress, leading to liver damage. TEO has been shown to possess potent antioxidant properties. Improved Liver Histopathology: Histopathological evaluation provides insights into liver tissue damage. Heydarian *et al.* [56] demonstrated that TEO administration improved liver histopathological parameters, indicating a potential protective effect against liver injury.

Conclusion

Chitosan Nano-Encapsulation is one approach that has the potential to improve the quality of quail meat while still keeping the components, qualities, and effects of the essential oils that are employed in low amounts.

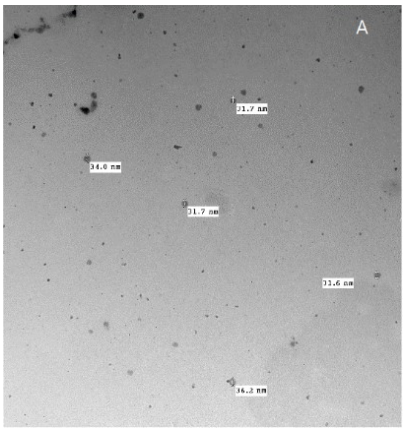

Fig. 1a. TEM of Capsulated 5 % thyme oil with nano-chitosan

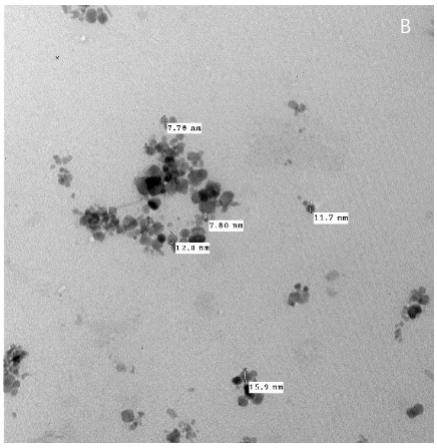

Fig .1b. TEM of Capsulated 3 % thyme oil with nano-chitosan

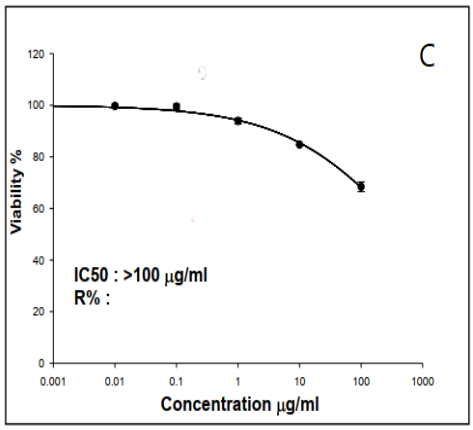

Fig. 1c. Cell viability % of Capsulated 5 % thyme oil with nano-chitosan effect on Vero cells.

TABLE 1. Composition and calculated analysis of the basal diet

Ingredients	**%**
Yellow corn	55.54
Soybean meal (44%)	35
Corn gluten meal (60%)	6.50
Dicalcium phosphate	0.80
Limestone	1.35
NaCl	0.35
Vit&Min premix[1]	0.30
DL. Methionine	0.05
L. Lysine hydrochloride	0.11
Total	100
Calculated analysis[2]	
Crude Protein %	24.02
Crude fiber%	3.87
Metabolizable energy, Kcal/kg	2900
Calcium%	0.81
available Phosphorous %	0.30
Methionine+Cystine %	0.75
Methionine %	0.50
Lysine%	1.30

[1]each 3kg of premix contains Vit. A, 10000.000 IU; Vit. D3, 2000.000 IU; Vit E, 35 g; Vit. K3, 2.5 g; Vit. B1, 2g, Vit. B2, 5g, Vit. B6, 2g; Vit. B12, 10g; Pantothenic acid, 15g; Nicotinic acid, 50g; Folic acid, 5g; Biotin, 200mg; Choline, 1000g; Copper, 5g; Iodine, 0.3g; Iron, 100g; Manganese, 70g; Zinc, 55g; Selenium,.2g.
[2]Calculated according to NRC [22].

TABLE 2. Effect of thyme essential oil alone or capsulated with nano-chitosan added in the diet on productive performance of Japanese quail.

Traits	BW(g) 7day (g/bird)	BW(g) 28 day (g/bird)	BW(g) 42day (g/bird)	BWG 7-42 day (g/bird)	FI 7-42day (g/bird)	FCR 7-42day (g/gain)
T1	25.9	155c	217	191.3	608.0^{c}	3.18
T2	25.6	163 b	220	196.8	627.5^{bc}	3.19
T3	25.7	168ab	229	199.4	657.0^{ab}	3.30
T4	25.8	173a	224	200.7	685.5^{a}	3.42
T5	26.0	169ab	223	196.9	646.8^{abc}	3.30
MSE	0.6	2.7	2.9	5.5	12.3	0.08
P-	NS	*	NS	NS	*	NS

[a,b,.....] Means within the same column with different superscripts are significantly differ ($P \leq 0.05$).NS: not significant
T1:Control, T2:control + thyme essential oil at 50 mg/kg diet, T3: control +thyme essential oil at 70 mg/kg diet, T4: control +thyme essential oil capsulated with Nano-chitosan at 30 mg/kg diet, and T5: control +Nano-chitosan at 50 mg/kg diet. BW: body weight, BWG: body weight gain, FI: feed intake, FCR: feed conversion ratio.

TABLE 3. Effect of thyme essential oil alone or capsulated with nanochitosan added in the diet on carcass characteristics.

Traits Treatment	Carcass %	Giblet %	Bursa %	Spleen %	Thymus %	Abd. fat %	Intestine weight%	Cecum length(cm)
T1	69.6	4.73	0.08	0.06	0.17	0.50	3.19	7.42^{b}
T2	68.7	4.88	0.07	0.06	0.27	0.76	3.28	8.96^{ab}
T3	71.6	4.44	0.08	0.06	0.36	0.49	3.37	9.53^{ab}
T4	66.8	4.44	0.09	0.05	0.25	0.55	3.47	10.20^{a}
T5	68.6	4.43	0.12	0.05	0.37	0.85	3.45	10.35^{a}
MSE	1.8	0.32	0.02	0.01	0.09	0.13	0.95	0.62
P-value	NS	NS	NS	NS	NS	NS	NS	*

a,b, Means within the same column with different superscripts are significantly differ ($P \leq 0.05$).NS: not significant

T1:Control, T2:control + thyme essential oil at 50 mg/kg diet, T3: control +thyme essential oil at 70 mg/kg diet, T4: control +thyme essential oil capsulated with Nano-chitosan at 30 mg/kg diet, and T5: control +Nano-chitosan at 50 mg/kg diet.

TABLE 4. Effect of thyme essential oil alone or capsulated with nano-chitosan on Proximate meat chemical Composition traits of quail.

TRAIT Treatment	MOISTURE%	ASH %	PROTEIN %	FAT %
T1	66.72	1.31	23.35	9.21
T2	66.67	1.04	22.99	9.20
T3	66.87	1.11	23.59	9.20
T4	68.85	1.07	22.53	8.72
T5	66.13	1.12	22.48	10.43
MSE	0.9	0.12	0.88	0.84
P-VALUE	NS	NS	NS	NS

$^{a,b,\cdots}$ Means within the same column with different superscripts are significantly differ ($P \leq 0.05$).NS: not significant.

T1:Control, T2:control + thyme essential oil at 50 mg/kg diet, T3: control +thyme essential oil at 70 mg/kg diet, T4: control +thyme essential oil capsulated with Nano-chitosan at 30 mg/kg diet and T5: control +Nano-chitosan at 50 mg/kg diet.

TABLE 5. Effect of thyme essential oil alone or capsulated with nanochitosan on meat quality traits of quail.

Trait **Treatment**	TBARS (mg of MDA/kg)	TVB-N (mg/100 g)	WHC %	Cooking loss %
T1	0.78^{a}	13.29^{a}	63.13^{d}	23.55^{a}
T2	0.66^{b}	6.51^{b}	65.80^{c}	22.63^{ab}
T3	0.56^{c}	5.92^{bc}	68.46^{b}	21.99^{b}
T4	0.46^{d}	5.35^{bc}	69.95^{a}	21.40^{bc}
T5	0.39^{d}	4.86^{c}	70.80^{a}	20.17^{c}
MSE	0.03	0.39	0.38	0.40
P-value	**	**	**	**

^{a,b,,c,d}… Means within the same column with different superscripts are significantly differ ($P \leq 0.01$).NS: not significant

T1:Control, T2:control + thyme essential oil at 50 mg/kg diet, T3: control +thyme essential oil at 70 mg/kg diet, T4: control +thyme essential oil capsulated with Nano-chitosan at 30 mg/kg diet, and T5: control +Nano-chitosan at 50 mg/kg diet. (2-TBARS): thio-barbituric acid reactive substances and total volatile basic nitrogen (TVB-N) and WHC: water holding capacity.

TABLE 6. Effect of thyme essential oil alone or capsulated with nanochitosan on serum constituents.

Traits treatment	Glucose (mg/dl)	T. protein (g/dl)	Globulin (g/dl)	Albumin (g/dl)	Albumin/Globulin ratio
T1	291.3	3.130 ^{ab}	1.624 ^{a}	1.506 ^{a}	0.95^{ab}
T2	295.5	3.835 ^{a}	2.030 ^{a}	1.805 ^{a}	0.71^{b}
T3	270.3	2.830 ^{ab}	1.345 ^{a}	1.485 ^{a}	1.2^{a}
T4	257.0	2.285 ^{b}	1.118 ^{a}	1.168 ^{a}	0.86^{ab}
T5	295.5	3.100 ^{ab}	1.377 ^{a}	1.057 ^{a}	1.15^{ab}
MSE	28.3	0.254	0.366	0.225	0.073
P	NS	*	*	*	*

$^{a,b,\ldots}$ Means within the same column with different superscripts are significantly differ ($P \leq 0.05$).NS: not significant
T1:Control, T2:control + thyme essential oil at 50 mg/kg diet, T3: control +thyme essential oil at 70 mg/kg diet, T4: control +thyme essential oil capsulated with Nano-chitosan at 30 mg/kg diet, and T5: control +Nano-chitosan at 50 mg/kg diet.

TABLE 7. Effect of thyme essential oil alone or capsulated with nanochitosan on lipid profile.

Traits treatment	Chol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	TG (mg/dl)
T1	177.400^{a}	84.300^{a}	70.660^{a}	155.200^{ab}
T2	177.250^{a}	83.300^{a}	80.150^{a}	181.500^{a}
T3	174.750^{a}	82.800^{a}	79.650^{a}	131.000^{ab}
T4	188.250^{a}	110.925^{a}	102.325^{a}	114.750^{b}
T5	150.000^{a}	93.500^{a}	56.500^{a}	102.833^{b}
MSE	0.909	0.215	0.626	0.029
P	*	*	*	*

$^{a,b,\ldots}$ Means within the same column with different superscripts are significantly differ ($P \leq 0.05$).NS: not significant
T1: Control, T2:control + thyme essential oil at 50 mg/kg diet, T3: control +thyme essential oil at 70 mg/kg diet, T4: control +thyme essential oil capsulated with Nano-chitosan at 30 mg/kg diet and T5: control +Nano-chitosan at 50 mg/kg diet. cholesterol(chol), high-density lipoproteins (HDL), Low-density lipoproteins (LDL), Very Low-density lipoproteins (VLDL), and triglyceride(TG).

Abbreviations

Essential Oils (EO)
Thyme Essential Oil (TEO)
Thyme Oil Nano Chitosan-Encapsulated (ETEO).
Treatment (T)
Milligram / Kilogram (Mg/Kg)
Thiobarbituric Acid Reactive Substances (TBARS)
Total Volatile Basic Nitrogen (TVB-N)
Total Antioxidant Capacity (TAC)
Low Density Lipoprotein (LDL)
Body Weight Gain (BWG)
Body Weight (BW)
Feed Intake (FI)
Crude Protein (CP)
Metabolizable Energy (ME)
Natural Killer Cells (NK Cells)
Feed Conversion Ratio (FCR)
Total Protein (TP)
Kilocalories (Kcal)
Average Body Weight Gain (ABWG)
International Unit (IU)
Gram (G)
Polydispersity Index (PDI)
High-Resolution Transmission Electron Microscopy (HRTEM)
SRB (Sulforhodamine B) Assay
Gas Chromatography–Mass Spectrometry (*GC–MS*)
Dulbecco's Modified Eagle Medium (DMEM)
Kelvin (Kev)
Milligram To Millilitre (Mg/Ml)
Micrograms Per Milliliter (Ug/Ml)
AOAC (Association Of Official Analytical Chemists)
Carbon Dioxide (CO_2)
Degree Celsius (°C)
Malondialdehyde (MDA)
Water Holding Capacity (WHC)
Centimeters (*Cm*)
Aspartate Aminotransferase (AST)
Alanine Transaminase (ALT)
Superoxide Dismutase (*SOD*)
Least Squares Mean (*LSM*)
Standard Error (*SE*)
Millisiemens Per Centimetre (*Ms*/*Cm*)
Dynamic Light Scattering (DLS)
Kidney Of An African Green Monkey (Vero Cells)
Half Maximal Inhibitory Concentration (IC50)
Abdominal Fat % (Abd. Fat %)
Milligrams Per Decilitre (Mg/Dl)
Grams Per Decilitre (G/Dl)
Cholesterol(Chol)
High-Density Lipoproteins (HDL)

Low-Density Lipoproteins (LDL)
Triglyceride(TG)
UA(Uric Acid)
CREAT (Creatinine)
Hepatic 3-Hydroxy-3-Methyle Glutaryl Coenzyme A (HMG-Coa)
Megavolt *(MV)*
Total Antioxidant Capacity (TAC).

Ethical statement

The study's experimental protocol had be approved by the Institutional Animal Care and Use Committee at Agricultural Research Center, Egypt under license number (ARC-AHRI: 2236). All methods had be carried out in accordance with the world Organization for Animal Health (OIE) and the Eighth Edition of the Guide for the Care and Use of Laboratory Animal (2011).

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing Interest

the authors have no competing interests to declar**e**

Funding sources

Not applicable

Authors' Contributions

Marwa Hosni Abd El- Maged and Hassan Abd El_ Krim Hassan Abd El-Halim were designed the experiment. Dalia Mohammed Ali Elmasry was synthesis and characterization of nanomaterials and wrote the nanomaterial section, Marwa Hosni Abd El- Maged and Hassan and Dalia Mohammed Ali Elmasry and Samah Ahmed Abdel-Tawab were conducted the experiment and data collection Amal Ahmed Abdel-Halim analyzed data and Marwa Hosni Abd El- Maged wrote the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to acknowledge and thank Prof. Dr. Mahmoud Arafa for his help in the scientific writing.

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تأثير كبسولة الشيتوزان النانومترية المغلف لزيت الزعتر على الإنتاجية وأداء النمو وجودة اللحوم لطائر السمان الياباني

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تناول هذا البحث مدى تأثير الخصائص الإنتاجية والفسيولوجية وجودة اللحوم بزيت الزعتر المغلف بالنانو شيتوزان (ETEO).

استهلكت مجموعة السيطرة (T1) العليقة الأساسية بدون مكملات، في حين غذت المجموعتان الثانية والثالثة مجموعة السيطرة بزيت الزعتر العطري (TEO) بجرعة 50 ملغم/كغم علف (T2) أو 70 ملغم/كغم علف (T3). وأخيرًا، تم تغذية المجموعتين الرابعة والخامسة بالتحكم + ETEO بجرعة 30 ملجم/كجم (T4) و50 ملجم/كجم (T5) حتى عمر 42 يومًا. بالمقارنة مع المجموعة الضابطة، كان لطيور السمان التي أعطيت نظامًا غذائيًا (T4) أكبر وزن للجسم (BW)، وتناول العلف (FI) في 28 يومًا، وتناول البروتين الخام (CP)، وتناول الطاقة المهضومة(ME) من 7 إلى 42 يومًا.

من حيث جودة اللحوم، كان لدى مجموعتي T4 وT5 مستويات TBARS أقل بكثير ($P < 0.05$) من المجموعة الضابطة والمجموعات الأخرى. كان لحم طائر السمان (الفخذ والصدر) لديه أقل كمية من النيتروجين الأساسي المتطاير (TVB-N) ($P < 0.05$)، في حين أن T4 كان لديه أقل كمية. أدت المكملات الغذائية إلى تحسين قدرة الدم الكلية لمضادات الأكسدة (TAC) في الطيور التي تم تغذيتها على (T3) مقارنة بـ (T2) والمجموعة الضابطة. أظهر كل من T2 وT3 انخفاضًا ملحوظًا في البروتين الدهني منخفض الكثافة (LDL) ($P < 0.05$). أدت التغذية T4 وT5 إلى زيادة مستويات TP وG بشكل كبير. الاستنتاج: يمكن أن يكون تغليف الشيتوزان النانوي طريقة للاحتفاظ بمركبات وخصائص وتأثيرات الزيوت العطرية المستخدمة بتركيزات منخفضة وتحسين جودة لحم طائر السمان.

الكلمات الدالة: طائر السمان، النمو، الصفات الفسيولوجية، جودة اللحم، كبسولة الشيتوزان نانوية لزيت الزعتر.