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Nano-emulsion Essential Oils Blend as a Green Alternatives to Antibiotics on Broilers Productive Performance

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Abstract

The current study was designed to evaluate the effect of supplementing different levels of nano-emulsion essential oils (NEEO's) blend (garlic and onion; 1: 1) on broilers performance, carcass traits, digestibility of nutrients, serum biochemical parameters and antioxidant responses. A total number of 200 unsexed d-old Arbor Acres broiler chicks were randomly distributed into 5 equal treatments containing 40 chicks each (10 birds /replicate). The first group fed standard diet and served as a control (T1). The second group was fed diet supplied with antibiotics (Oxytetracyclinne; 50 mg Kg-1 diet) (T2). Other three groups T3-T5 fed basal diet supplemented with 2.5 NEEO's, 5 NEEO's and 10 NEEO's ml kg-1 diet nano-emulsion essential oils blend (garlic and onion), respectively for 35-day. At the end of the feeding trial, the results showed that compared to the control chicks of T4 significantly recorded the best EE% digestibility coefficient, while crude fiber (CF%) digestibility coefficient was significantly decreased by adding different supplementation. The highest body weight gain (BWG) during overall growth period was achieved by chicks fed 2.5 ml NEEO's kg-1 diet, while, the best feed conversion ratio (FCR) was found in chick fed 5 ml NEEO's kg-1. Chicks fed 10 ml NEEO's kg-1 diet recorded the lowest intestinal bacterial count, lipid profile values and total saturated fatty acids in both breast and thigh muscles. The highest antioxidant values, immune status also breast and thigh mono and poly-unsaturated fatty acids were achieved in 10 ml NEEO's kg-1 diet group.

Keywords: Garlic; Onion; Essential Oil; Nano-emulsion; Performance; Broiler.

Introduction

The poultry industry, which is also the fastest growing farming sector and a major economic force, is leading the way in meeting consumer demand for meat and eggs as well as improving the nutritional value of human food. The sector has become more well-known because of its quick outcomes (Abd El-Hack *et al.*, 2024). High animal productivity is ensured when poultry feed has readily available, high-quality ingredients that meet demand.

Sub therapeutic antibiotic doses have mostly been used in the broiler sector to promote growth performance with minimizing mortality and morbidity (Abd El-Hack *et al.*, 2020). Antibiotic-resistant bacteria are more likely to arise in the poultry industry due to the extensive use of antibiotics, which creates a selective environment (Abreu *et al.*, 2023). Chicken products that contain antibiotic residues, like meat or eggs, are harmful to human health since these substances lead to the development of antibiotic resistance in pathogenic microbes that are present in human flora (Mancuso *et al.*, 2023).

However, the European Union has forbidden antibiotics due to the presence of residues of antibiotics in broiler products, the significant rise in the formation of resistant bacteria to antibiotics (Namdeo *et al*., 2020). Therefore, scientists have been encouraged to examine potential alternatives to antibiotics for application in chicken feeds in order to prevent negative antibiotic impacts, promote consumer safety, and protecting the ecosystem (Alagawany *et al.*, 2021). The use of nutritional supplements, such as probiotics and prebiotics, has been studied during the past 20 years as a potential antibiotic substitute (Abd El-Hack et al., 2020).

A widespread belief that phytogenic compounds have high nutritional value has led to a considerable deal of attention being paid to them. Phytogenic feed additives, which include essential oils, are effective alternatives for increasing meat production efficiency and improving broiler performance (Namdeo *et al*., 2020).

Garlic essential oil (*Allium sativum L.*) includes flavones,
terpenoids, quinine, tannins, saponins, esters,
polyphenols, flavonoids ,alkaloids and volatile

residues. These compounds have a wide range of beneficial properties, including anti-coccidail, antioxidant, antibacterial and gastrointestinal enzyme enhancers, which improve nutritional utilization, digestion, absorption, and liver function (Abdallah *et al.,* 2022).

Onion (*Allium cepa*) seeds essential oil is distinguished by the flavanol quercetin component and its derived compounds, which give onion their special flavor. It additionally contains a variety of other bioactive compounds, including sulphur compounds and fructo-oligosaccharides (Roldán *et al.,* 2008). The sulphur contents serve as the basis for the specific antibacterial and antioxidant activities (Kim *et al*, 2004).

Nanoemulsion is colloidal dispersions of two immiscible liquids like oil, surfactant, and cosurfactant with different ratios. One of the safest applications of nanotechnology in animal nutrition is nano-emulsified essential oils, in which the size of oil droplets ranges from 20 to 100 nanometers. (Abdallah *et al.,* 2022). Nano emulsions are a medium that increases the biological absorption of essential oils. Nano emulsions are commonly used in the food sector to encapsulate, preserve, and deliver biologically active substances with low solubility in water (Rao and McClements, 2011). In addition, Nano emulsion may encapsulate numerous lipophilic substances, increasing their absorption rate (Acosta, 2009). As a result, we consider antibiotic substitutes, such as plant-derived essential oils and nano emulsions, as innovative antibiotic alternatives.

The nano-emulsified essential oils were more effective in raising fatty acids (n-3 and n-6) and lowered the bio hydrogenation rate of polyunsaturated fatty acids to saturated fatty acids (Abdallah *et al*., 2022; Mousa *et al*., 2022). Nano-emulsified oil supplements were more effective than raw oil supplements (Mohamed and Abd El-Wahab, 2022). In this context, Abudabos et al. (2021) found that t adding 5ml/L of nano-emulsified vegetable oil for broiler chick diets and reared under heat stress, enhanced their performance and meat quality. Furthermore, AL-Ramamneh (2017) evaluated the effect of adding of onions extract to drinking water with different levels (0, 2.5, 5, and 7.5%) on broiler performance, the researcher observed that by raising the onion level, the weight gain was increased.

In the majority of earlier investigations, the focus was on the effect of individual essential oil on performance and immunology; however, little reports were not found to clarify the effect of blended essential oils on the performance of broiler. Therefore, the objective of the current study was to evaluate the effects of adding Nano- emulsified essential oil blend (garlic and onion) as feed additive, on growth performance, blood components, immunity indices, meat analysis and meat quality of broiler chicks.

Materials and Methods

This experimental work was conducted at the Poultry Research Farm of the Animal Production Department, Faculty of Agriculture, Moshtohor, Benha University. Chemical analysis was also carried out at the Animal Production Department, Faculty of Agriculture at Moshtoher, Benha University, and the Food Analysis Centre, Faculty of Veterinary Medicine, both of which are affiliated with the same university, from 17 September to 22 October, 2022.

2.1. Essential garlic and onion oil and surfactant

Garlic and onion oils were provided from the National Research Centre (NRC), Giza, Egypt. Tween 80 was provided from Sigma Aldrich Company, and water was distilled just before using.

2.2 Preparation of garlic and onion nano-emulsified essential oil blend

The Nano emulsion was prepared in animal production department lab, Faculty of Agriculture, Benha Uni, Egypt according to methos of Ragavan *et al.,* (2017) with some modification. Briefly, mixing 10 ml of garlic and onion oil at ratio (1:1) with 10 ml of Tween 80 as surfactant, then added 80 ml distilled water. After mixing, the materials were homogenized for 30 min. using homogenous blender powered (1500 watt), then gradually adding distilled water to the combined oil phase.

2.3 Characterization of garlic and onion nano-emulsified essential oil blend

2.3.1 Zeta Potential

The formed oil Nano emulsions have been characterized using the Zetasizer Instrument. This apparatus measures oil droplet size, the surface charge (zeta potential), distribution of size, and electric conductivity. In order to prevent oil particles from re-aggregating for as long as possible, the charge on the surface of the Nano emulsion formation was measured using Laser Doppler electrophoresis (SZ-100- Horiba Scientific, - Kyoto., Japan). The samples were diluted with deionized water before being injected into a tiny capillary cell to test the charge at 25 C°. Zeta potential values were measured in millivolts, and the result was an immediately apparent zeta potential image (Figure1).

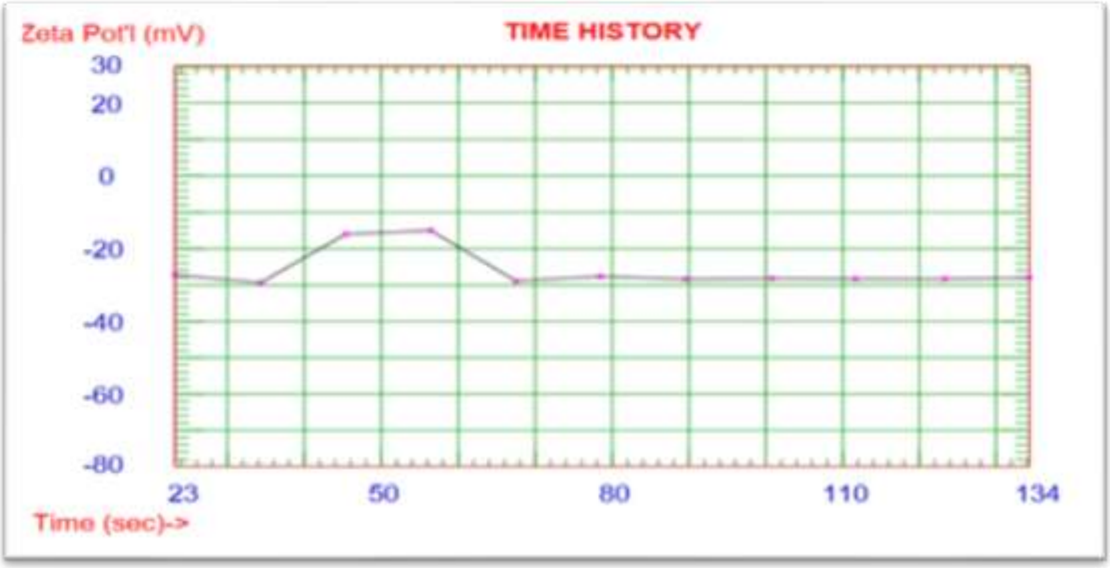

Figure 1: garlic and onion nano- emulsified essential oil blend Zeta Potential

2.3.2 Transmission electron microscopy (TEM)

The structure and form of the garlic and onion nano- emulsified essential oil blend were investigated using a TEM (FEI-TECNAI G2- 20 TWIN, Netherlands). The nano-emulsions were diluted with water that had been deionized at 10- and 100-fold tiny drops before being applied on 300 mesh copper grids covered with carbon film. After drying in vacuum for three hours, the grid was TEM examined at 80 kV, showing an excellent picture (Figure 2).

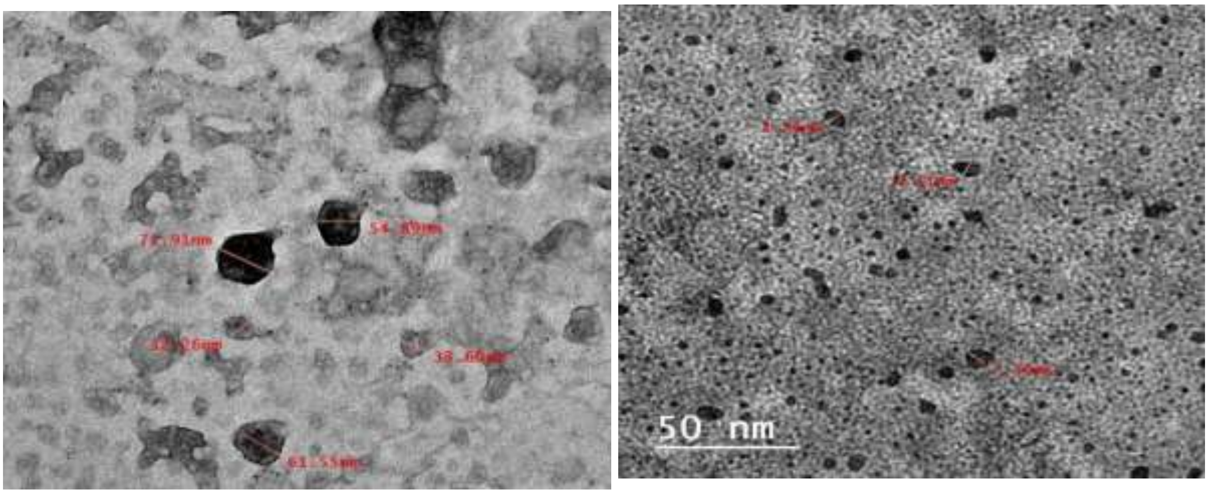

Figure 2. Transmission electron microscopic (TEM) images of nano emulsified essential oils blend (garlic, and onion essential oil).

2.4 Birds, diets, and experimental design

Two hundred Arbour Acres broiler chicks weighing an average of (40±5 g) were randomly assigned to five experimental groups, each including forty birds (every one group was divided into four replicates, ten birds each). The first group (T1) was served as a control and the second group (T2) was fed the control diet supplementing with Antibiotic (Oxytetracyclinne 50 mg/Kg diet), the three other groups were fed on 0.25, 0.50 and 1 ml/kg diet oil blend (garlic, and onion) as T3 (2.5 ml NNEO's), T4 (5 ml NNEO's) and T5 (10 ml NNEO's) groups, respectively. The basal diet was formed to provide Arbour Acres broilers with the nutrients they needed (Table 1). The poultry feed was formulated and presented in the form of a fine mixture, The mashed chicken feed was immediately sprayed with various dosages of nano emulsion, dried, and kept in well-ventilated and a clean area until needed for the experiment. Water and feed were given out on *ad libitum*. Every chick was inoculated for common diseases, kept in identical management, hygienic, and ideal environmental conditions, and received regular observations. At the beginning and ending of each growth phase, live body weight (LBW) and feed intake (FI) were recorded separately. Body weight gain (BWG) and the feed conversion ratio (FCR) were also calculated.

Table 1. Composition of the basal diets used in this study

Ingredients	Starter diet (1-10 days)	Grower diet (11-22 days)	Finisher diet (23-35 days)
Yellow corn	51.63	57.69	**63.05**
Soybean meal (44%)	34.52	27.14	**22.85**
Corn gluten meal (60%)	6.32	7.55	**6.70**
Soybean oil	3.00	3.50	**3.50**
Di calcium phosphate	1.84	1.63	**1.51**
Limestone	1.27	1.17	**1.14**
Common salt	0.40	0.40	**0.40**
Sodium bi carbonate	0.10	0.10	**0.10**
Vit. & min. mix.*	0.30	0.30	**0.30**
DL-Methionine	0.25	0.18	**0.15**
L- Lysine HCL	0.37	0.34	**0.30**
Total	100	100	**100**
Calculated analysis**			
CP %	23.1	21.10	**19.10**
ME (kcal/kg)	3006	3128	**3200**
CF %	3.74	3.35	**3.14**
EE %	5.46	6.15	**6.29**
Calcium %	1.05	0.95	**0.90**
Available Phosphorus %	0.50	0.45	**0.42**
Methionine %	0.68	0.59	**0.53**
Lys. %	1.49	1.28	**1.12**

* Vitamin A (12 000 000 IU), D3 (4 000 000 IU), E (50 g), K3 (3 g), B1 (3 g), B2 (7 g), B6 (4 g), B12 (20 mg), Nicotinic acid (50 g), Pantothenic acid (15 g), Biotin (150 mg), Folic acid (2 g), Choline (500 g),Copper (10 g), Iron (30 g),Manganese (100 g) , Zinc (80 g),Selenium (0.3 g), , Iodine (1.25 g), Cobalt 0.1 g, and carrier (CaCO3) up to 3 kg are all included in each 3 kg. ** According to data provided by the Regional Centre for Food and Feed in Egypt (**RCFF, 2001**).

2.5 Digestibility trial

At the end of the finisher stage, 20 chicks (four per treatment) had been used for determining the nutrients digestibility coefficients. The experimental diets and water were provided ad libitum, while excreta had been collected and sprayed using 1% boric acid in order to prevent ammonia losses, then dried at 60°C for 24 hours. A chemical approach was used to separate the nitrogen content of excreta into urine and feces (Jakobsen *et al*., 1960). Diets and dried excreta had been analyzed according to (AOAC, 2000) including dry matter (DM), organic matter (OM), crude protein (CP), crude fiber (CF), ether extract (EE) and nitrogen free extract (NFE) at the Food Analysis Centre, Faculty of Veterinary Medicine, Benha University.

2.6 Slaughtering trial and meat quality

At the end of the 35-day trial, the slaughter evaluate was conducted. Four birds were randomly selected from each test group. The birds were starved for 12 hours before slaughter, then individually weighed at the nearest gramme (pre-slaughter weight) and slaughtered using the standard Islamic tradition procedure. Following complete bleeding and feathers pecking, the empty carcass, heart, gizzard, abdominal fat, liver, and lymphoid organs (bursa, thymus and spleen) were taken out and weighed. The percentages were then determined for all previous parts based on the pre-slaughter weight.

Forty samples (4 birds per treatment) were collected for chemical analysis, including moisture %, fat%, protein%, and ash %) for both (breast and thigh muscles), in addition to fatty acid fractionation in accordance with the AOAC (2000).

2.7 Blood parameters

During the slaughter trial, four bird blood samples were collected from each group to represent each experimental group. Each chicken had five milliliters of blood collected into a clean, dry tube with no use of anticoagulant, To obtain blood serum, then samples were centrifuged at 3500 rpm for 20 minutes, then serum was separated into 2-ml samples and put into dry, clean eppendorf tubes and kept at 20 °C for analysis of total protein according to (Armstrong and Carr, 1964) and albumin according to (Doumas *et al*., 1971). Subtracting determined albumin from total protein gave the globulin value. Richmond (1973) estimated the following total cholesterol (mg/dl), low density lipoprotein (LDL; mg/dl), and high density lipoprotein (HDL; mg/dl). An enzymatic colorimetric approach was used to test blood urea nitrogen, according to Patton and Cronch (1979). Using easily accessible commercial kits from Biomerieux, France, creatinine were measured using the Julian (2000) authorized technique and expressed as (mg/dL).

Alanine aminotransferase (ALT) and the aspart ate aminotransferase (AST) were measured and reported as IU/L in accordance with Reitman and Frankel's (1975) methodology. The concentrations of immunoglobulin M (IgM) and immunoglobulin G (IgG) in the Bird's serum were measured using standard bio diagnostic kits from (Bio Diagnostic Company -Giza, Egypt) and an ultraviolet (UV) spectrophotometer, Shimadzu, Japan. Wang *et al.*, (2011) specified that Malondialdehyde (MDA) bird's serum was measured and expressed by U/mg protein, which is a byproduct of lipid peroxidation. The techniques outlined by Wang *et al.*, (2011) were used to assess the activities of superoxide dismutase (SOD). According to Fang *et al.*, (2011), the antioxidant capacity of glutathione peroxidase (GPx) was measured commercially using GPx kits (Randox, Crumlin, UK) and represented as unit/mg protein.

2.8 Small intestine total microbial count

Four individual birds from each treatment group (the exact same birds used for the slaughter test) had their small intestine contents removed under aseptic conditions. These contents were then used to count the microbial populations in the duodenum, jejunum, and ileum of the birds using a variety of dehydrated media (Biolife Ltd, Italy). Fernando *et al.*, (1994) plate count methods were used to count the microbial populations.

2.9 Economic Efficiency

The quantity of feed utilized throughout each stage of the entire experiment was calculated in order to calculate the relative economic efficiency (REE) for white meat production. To get the overall feed cost, the total feed intake per bird was multiplied by the cost of one kilogram of each experimental diet. This, at the time of the experiment, was approximated using current local prices. During the whole experimental period, the economic efficiency of growth (EEG) was determined using the following formula: EEG=100× [(market price per total gain – total feeding cost) / total feeding cost].

2.10 Statistical analysis

Before analysis, data were subjected to homogeneity and normality tests (Ewens and Grant, 2005). Following that, the SAS ANOVA technique was used to analyze all the data (SAS, version 6.03, Soft Inc., Tusla, OK, USA, SAS, 2013). To ascertain whether there was appreciable variation among the treatments, a one-way ANOVA was utilized. When overall variations were detected, Duncan's (1955) test was used to examine the variances between the means. The individual impacts of two different densities (Low and High), as well as different concentrations of blended nano emulsion essential oils, were examined using a one-way ANOVA. Results are shown ±MSE, the differences were deemed significant at $P < 0.05$.

Results and Discussion

3.1 Nutrient digestibility coefficients

Effect of Nano-emulsified essential oils blends (garlic and onion) on digestibility of various nutrients are listed in Table 2. Chicks fed different treatments did not show any significant ($P < 0.05$) effect on CP, NFE and OM digestibility. While, supplementation of different levels of NEEO's significantly improved digestibility of ether extract by 1.77%, 3.53% and 2.93%, respectively compared to control.

Table 2. Effect of different treatments on nutrient digestibility coefficients:

Treatments	CP %	EE %	CF%	NFE %	OM %
T1(Control)	91.54	69.33 [e]	35.21 [a]	84.61	**89.24**
T2 (Oxytetracyclinne 50 mg)	92.20	70.97 [c]	24.39 [c]	84.66	**89.27**
T3 (2.5 ml NEEO's)	94.28	70.56 [d]	26.28 [b]	74.63	**89.25**
T4 (5 ml NEEO's)	94.32	71.78 [a]	21.32 [d]	84.63	**89.25**
T5 (10 ml NEEO's)	92.57	71.36 [b]	19.45 [e]	84.64	**89.26**
±SE	3.42	0.027	0.065	0.014	**0.01**
P value	0.966	0.0001	0.0001	0.289	**0.226**

The means of rows a, b,.. e with different superscripts shows a significant difference ($P<0.05$).

Regarding to CF digestibility, chicks fed control diet recorded the highest value (35.21%) followed by group of T3 (26.28%), then T2 (24.39%), T4 (21.32%) and the worst group was for T5 (19.45%). Lower percentage of CF digestibility may be due to the antibacterial effect of garlic (Cheng *et al.,* 2016) and onion (Joe *et al.,* 2009) oils which have antibacterial effect which in term affect the bacterial population in the gut (as well be mentioned later in Table 6), these bacterial are responsible of fiber digestion. These findings are in agreement with Issa and Abo Omar (2012) who stated that apparent digestibility of EE for broiler was significantly improved ($P < 0.05$) by the addition of the garlic powder compared to that in the control diet. Also, Abdallah *et al*., (2022) found that when weaned Gabli rabbits were fed diet supplemented with nano-emulsified essential oil blend (lemon, garlic and onion) the digestibility coefficients of CP, EE, and NFE were significantly ($P <0.05$) increased when 10 ml/kg, 7.5 ml/kg, and 5 ml/kg of the blend were added, compared to the control.

3.2 Growth performance:

Table 3 show that chicks of 2.5 ml NEEO's group achieved the highest final live weight and body weight gain without significant differences to others of 5 NNEO's and 10 NNEO's groups, while the lowest values were recorded for control group. Broilers fed diet supplemented with 5 NEEO's recorded the lowest FI (2521.75 g) without significant difference to groups fed diets supplemented with either antibiotics (T2) or 10 NEEO's (T5) being 2715.5 g and 2716 g, respectively. Then chicks fed 2.5 NEEO's added group (2766.75 g), while group fed basal diet recorded the highest FI value (3120.25 g). Feed conversion ratio was improved in group of T4 (1.36) without significant difference to T3 (1.41) and T5 (1.42), whereas, with significant difference to dietary antibiotic group (T2) being 1.50 and the worst FCR value was found in control group (T1; 1.79). The improvement in growth and feed utilization in the present study may be due to garlic has growth promoting properties in animals, which could be responsible for the improved growth performance of chicks, fed dietary NEEOs. Also, it could boost how effectively broiler feed intake, digest the feed, and absorb nutrients, which would improve their growth performance (Demir *et al*., 2003). Furthermore, onions have a variety of bio-active substances, including phenolic and flavonoid chemical substances, which have been positively associated with growth promoting the animal performance. According to Rahman *et al*. (2022), these substances may enhance the metabolism, nutrient absorption, and growth performance of broiler chickens. According to previous studies, adding EOs to the feed enhances growth performance by inducing the release of digestive enzymes, which increases nutrient absorption and digestion and also improving the intestinal passage rates (Jamroz *et al.*, 2005). Furthermore, it has been proven that adding EOs to chicken feed improves the activity of the enzymes trypsin and amylase (Jamroz *et al.*, 2005). It is theoretically possible to improve growth performance by including EOs, which lower the incidence of intestinal disorders caused by undesired bacteria and promote the formation of healthy gut microbiota (Bento *et al.*, 2013). Furthermore, Aji *et al*., (2011) observed that broilers fed diets contained freshly onion bulbs had higher body weight and FCR than broilers on a standard diet.

Table 3. Effect of different treatments on growth performance

Treatments	IW[1] (g)	FW[2] (g)	WG[3] (g)	FI[4] (g)	FCR[5]
T1(Control)	43.75	1782.50 [c]	1738.75 [c]	3120.25 [a]	**1.79 [a]**
T2 (Oxytetracyclinne 50 mg)	44.00	1851.75 [bc]	1807.75 [bc]	2715.50 [bc]	**1.50 [b]**
T3 (2.5 ml NEEO's)	43.75	2009.00 [a]	1965.25 [a]	2766.75 [b]	**1.41 [bc]**
T4 (5 ml NEEO's)	43.75	1891.50 [b]	1847.75 [abc]	2521.75 [c]	**1.36 [c]**
T5 (10 ml NEEO's)	44.00	1953.50 [ab]	1909.50 [b]	2716.00 [bc]	**1.42 [bc]**
±SE	0.85	48.24	48.26	65.61	**0.04**
P value	0.998	0.038	0.039	0.0002	**0.0007**

The means of rows a, b,.. e with different superscripts show a significant difference (P<0.05).
IW= Initial weight; FW = Final weight; WG = Weight gain; FI = Feed intake; FCR = Feed *conversion ratio*

3.3 Carcass trail:

Results of carcass characteristics and lymphoid organs as affected by different treatments are presented in Table (4), it is clear to note that none of the dietary groups influence any of the carcass parameters as mentioned by carcass%, liver%, heart%, gizzard% and abdominal fat.

Table 4. Effect of different treatments on carcass characteristics

Treatments	Carcass characteristics					Lymphoid organs		
	Carcass%	Liver %	Heart %	Gizzard %	Ab. fat	Spleen%	Bursa %	**Thymus %**
T1(Control)	77.12	2.22	0.51	0.87	0.97	0.14	0.12	**0.32 [b]**
T2 (Oxytetracyclinne 50 mg)	73.68	2.57	0.51	0.77	0.87	0.17	0.16	**0.44 [ab]**
T3 (2.5 ml NNEO's)	72.93	2.18	0.52	0.71	0.93	0.18	0.14	**0.47 [ab]**
T4 (5 ml NNEO's)	77.91	2.37	0.54	0.96	0.81	0.15	0.16	**0.60 [a]**
T5 (10 ml NNEO's)	75.60	2.39	0.48	0.86	1.02	0.17	0.14	**0.40 [ab]**
±SE	1.91	0.27	0.04	0.06	0.12	0.03	0.02	**0.05**
P value	0.33	0.844	0.784	0.113	0.74	0.81	0.733	**0.02**

The means of rows a, b,.. e with different superscripts show a significant difference (P<0.05).

This result agree with the previous research of El-Gogary *et al.* (2018) who tested four levels of raw garlic oil (0, 0.25, 0.5 and 0.75 g/kg) on growing rabbits, they noted that all experimental groups did not affected carcass, liver and giblets percentages. Regarding to lymphoid organs, various dietary groups did not significantly affect either spleen or bursa percentages, while group of 5 NEEO's significantly recorded the highest value of thymus % (0.6%) and control group recoded the lowest value (0.32%) whereas the other groups were within these values.

3.4 Meat chemical analysis and quality:

Effect of different treatments on breast and thigh chemical quality are illustrated in Table 5, in respect of breast chemical quality, moisture% and ash % did not significantly (P > 0.05) affected by different treatments studied. On the other hand, chicks fed diet 5 achieved the highest percentage of protein without significant differences to groups of antibiotic (T2) and 5 NEEO's (T4) while the lowest protein% was for control group (T1). Fat percentage significantly decreased by adding different dietary supplementations, where, groups of T2 (1.55%), T4 (1.82%) and T5 (1.42%) recorded significantly lower fat % than control (2.82%) and T3 (2.30%).

Regarding to thigh chemical quality, all experimental groups did not show any differences in thigh moisture%, group of antibiotics (T2) and 10 NEEO's (T5) recorded the highest value of protein and ash without significant differences to groups of 2.5 NEEO's (T3) and 5 NEEO's (T4) while the worst values was for control group (T1). Chicks fed control and dietary 2.5 NEEO's showed the highest thigh fat % and chicks fed dietary 10 NEEO's showed the lowest value. This result might be attributed to the fact that garlic essential oil and onion essential oil supplementation has been associated to better meat quality characteristics in broilers, resulting in greater satisfaction among consumers.

Table 6. showed the results of meat quality of breast. The lowest value of pH, , total volatile nitrogen (TVN) and thiobarbituric (TBA) were detected in chicks fed diet supplemented with 10 ml NEEO's followed by diet supplemented with 50 mg oxytetracyclinne, but the control diet recorded the highest levels.

Meat qualities of thigh of chicken as affected by different experimental group are listed in Table 7. Compared with control group, values of pH, TVN and TBA were significantly (P < 0.05) reduced by increasing levels of NEEO' (2.5, 5 10 ml). Furthermore, addition of 50 mg oxytetracyclinne decreased the values of pH, TVN and TBA compared with the control group. The lowest values of pH, TVN and TBA of were detected in chicken fed diet supplemented with10 ml NEEO's.

Abdallah *et al.*, (2022) showed that supplementing of 5, 7.5 and 10ml/kg diet nano-emulsified essential oil blend (NEOB) (garlic, onion and lemon essential oil) as a feed additive on weaned mountain rabbits. Recorded the highest values of meat protein compared with the control. While, meat pH, TVN and TBA the control group was higher values compared with 10ml/kg, 7.5ml/kg and 5ml/kg Nano emulsified essential oil blend.

Table 5. Effect of different treatments on chemical meat analysis

Treatments	**Breast**				**Thigh**			
	Moisture%	Protein%	Fat%	Ash%	Moisture%	Protein%	Fat%	**Ash%**
T1(Control)	73.07	19.90 ^{c}	2.82 ^{a}	2.67	73.42	18.97 ^{b}	3.17 ^{a}	**2.25^{b}**
T2 (Oxytetracyclinne 50 mg)	72.70	21.25^{ab}	1.55 ^{c}	3.20	73.10	20.45 ^{a}	2.20^{b}	**2.82^{a}**
T3 (2.5 ml NNEO's)	72.60	20.57^{bc}	2.30 ^{b}	2.87	73.72	19.50 ^{ab}	2.90 ^{a}	**2.45^{ab}**
T4 (5 ml NNEO's)	72.92	20.80^{abc}	1.82 ^{c}	3.10	73.50	19.92 ^{ab}	2.62^{ab}	**2.65^{ab}**
T5 (10 ml NNEO's)	72.4	21.72^{a}	1.42 ^{c}	3.32	72.90	20.60 ^{a}	2.02^{c}	**2.92^{a}**
±SE	0.26	0.34	0.13	0.16	0.25	0.36	0.18	**0.15**
P value	0.42	0.02	0.0001	0.08	0.18	0.03	0.002	**0.04**

The means of rows a, b,.. e with different superscripts show a significant difference (P<0.05).

Table 6. Effect of nano-emulsified essential oils blend on Chicken Breast meat quality

Treatments	**Meat quality of breast**			
	PH	**TVN %**	**TBA mg kg^{-1}**	**PVmeqO2 kg**
T1(Control)	5.71^{a}	2.85^{a}	0.12^{a}	**0.34^{a}**
T2(Oxytetracyclinne50 mg)	5.60^{ab}	1.78^{b}	0.07^{b}	**0.19^{b}**
T3 (2.5 ml NEEO's)	5.66^{a}	2.14^{b}	0.11^{a}	**0.29^{a}**
T4 (5 ml NEEO's)	5.63^{ab}	2.08^{b}	0.08^{ab}	**0.22^{ab}**
T5 (10 ml NEEO's)	5.57^{abc}	1.43^{b}	0.05^{bc}	**0.15^{bc}**
±SE	0.02	0.16	0.01	**0.03**

The means of rows a, b,.. e with different superscripts show a significant difference (P<0.05).

Table 7. Effect of nano-emulsified essential oils blend on Chicken thigh meat quality

Treatments	Meat quality of breast			
	PH	TVN %	TBA mg kg	PVmeqO2 kg
T1(Control)	5.75[a]	3.13[a]	0.14[a]	**0.41[a]**
T2(Oxytetracyclinne 50 mg)	5.64[ab]	2.07[b]	0.09[ab]	**0.25[ab]**
T3 (2.5 ml NEEO's)	5.71[a]	2.71[a]	0.12[a]	**0.34[a]**
T4 (5 ml NEEO's)	5.67[ab]	2.38[ab]	0.11[ab]	**0.32[ab]**
T5 (10 ml NEEO's)	5.60[abc]	1.69[bc]	0.07[abc]	**0.20[abc]**
±SE	0.03	0.17	0.01	**0.04**

3.5 Intestinal bacterial count and some blood parameters:

Results shown in Table 8 revealed that intestinal total bacterial count was reduced in group of 10 ml NEEO's (2.33×10^{12}) compared to control and other treatments. This result might be related to the fact that garlic contains bioactive substances, such as allicin, which have been found to have antibacterial activity against a variety of infections. Adding nano-emulsion essential garlic oil to chickens' gastrointestinal tracts could potentially reduce the growth of harmful pathogens (El Oksh *et al.*, 2022). Furthermore, onion essential oil has been shown to have antibacterial activity against certain infections. It may assist to reduce bacterial contamination in broiler diets (Chowdhury *et al.*, 2017). In this regard, Bokaeian and Bameri (2013) and Dangana *et al.*, (2016) reported that garlic has significant antimicrobial activity against the coliform bacteria and is effective in eliminating enterococci. Control group recorded significantly the lowest values of serum total protein, albumin and globulin while the highest values were for 10 ml NEEO's group. Concerning to kidney and liver functions, all experimental groups significantly improved either kidney or liver functions as measured by urea, creatinine, AST and ALT compared to control group.

Table 8. Effect of different treatments on serum biochemical parameters

Treatments	Serum biochemical parameters							
	Total bacterial count (CFU/g)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Urea (mg/dl)	Creatinine (mg/dl)	AST (u/l)	**ALT (u/l)**
T1(Control)	1.18×10^{13}	3.67[c]	2.22[c]	1.45[c]	27.35[a]	0.92[a]	56.47[a]	**32.87[a]**
T2 (Oxytetracyclinne 50 mg)	3.12×10^{13}	4.87[ab]	2.80[ab]	2.07[ab]	20.17[c]	0.57[c]	41.15[c]	**24.62[bc]**
T3 (2.5 ml NNEO's)	1.23×10^{13}	4.15[bc]	2.47[bc]	1.67[bc]	24.37[b]	0.77[b]	48.77[b]	**30.20[a]**
T4 (5 ml NNEO's)	2.33×10^{13}	4.47[b]	2.62[b]	1.85[b]	21.82[c]	0.71[b]	44.37[b]	**26.95[b]**
T5 (10 ml NNEO's)	5.1×10^{12}	5.47[a]	3.15[a]	2.32[a]	17.67[d]	0.49[c]	36.17[b]	**22.07[c]**
±SE	----------	0.23	0.15	0.11	0.74	0.04	2.42	**0.93**
P value	----------	0.0007	0.007	0.0007	0.0001	0.0001	0.0003	**0.0001**

The means of rows a, b,.. e with different superscripts show a significant difference ($P<0.05$).

3.6 Serum lipid profile, antioxidant status and immune response:

Effect of experimental treatments on serum lipid profile, antioxidant status and immune response is listed in Table 9, it is noticeable that by increasing nano-emulsified oils levels, the values of lipid profile (triglycerides, total cholesterol, HDL and LDL) decreased. The results obtained agree with previous research by Issa and Abo Omar (2012), who reported that nutritional supplementation with garlic powder at both doses (1.5% and 3.0%) resulted in a significant reduction in mean value of total cholesterol levels when compared with control broilers.

Similar results were reported by Prasad *et al.*, (2009), who found that garlic supplementation significantly lowered total cholesterol, LDL, VLDL, and triglycerides while significantly raising HDL in chickens up to 8 weeks old when compared to the control group. This could be due to the mechanism of garlic products' hypolipidemic and hypocholesterolaemic a ction, which reduces the hepatic processes of lipogenic and cholesterogenic enzymes among them glucose-6-phosphatase dehydrogenase, malic enzyme and fatty acid synthase, and 3-hydroxyl-3-methyl-glutaryl-CoA (HMG-CoA) reductase (Qureshi *et al.*, 1983a and Qureshi *et al.*, 1983b).

Regarding to antioxidant parameters, all experimental groups enhanced antioxidant enzymes (GPx and SOD) comparing to control hence the groups of 10 ml NEEO's achived the best values for both enzymes. On opposite MDA value was the lowest for 10 ml NEEO's group and the highest value was for control. This finding can be explained by the fact that garlic essential oil, which has antioxidants and other bioactive substances, might help lower lipid oxidation and oxidative stress in broiler meat (Chowdhury *et al.*, 2018). When onion essential oil was added, the same response was observed (Chowdhury *et al.*, 2017). The findings corroborate those of El-Gogary *et al.*, (2018) who reported that a group fed a diet containing 0.5 gram of garlic oil per kilogram enhanced their antioxidant status overall and reduced their MDA levels.

Immune response (Table 9) as mentioned by IgG and IgM were improved by adding different feed additives compared to control. While, the best group is 10 ml NEEO's which recorded 43.40 mg/dl and 66.12 mg/dl, respectively. In this respect, Garlic and onion essential oils compounds have been reported to possess immunomodulatory properties, enhancing the immune response in animals. By supplementing nano-emulsion essential of either garlic oil (Chowdhury *et al.*, 2018) or onion oil (Rahman *et al.*, 2022), It may boost the broiler chickens immune systems, improving their resistance to disease and general health. El-Gogary *et al.*, (2018) came to the conclusion that, in comparison to other groups (0, 0.25, and 0.75 g/kg), supplementing raw garlic oil at a level of 0.5 g/kg boosted (both IgG and IgM levels in growing rabbits.

Table 9. Effect of different treatments on blood lipid profile, antioxidant status and immune response

Treatments	Lipid profile				Antioxidant parameters			Immune response	
	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	GPx (U/L)	SOD (U/L)	MDA (mmol/mL)	IgG (mg/dl)	**IgM (mg/dl)**
T1(Control)	61.22[a]	88.90[a]	58.35[a]	30.55[a]	324[c]	203.25[c]	2.02[a]	29.72[c]	**50.47[d]**
T2 (Oxytetracycline 50 mg)	43.40[c]	62.05[d]	44.37[c]	17.67[c]	406.50[ab]	268.75[a]	1.42[bc]	39.85[a]	**59.30[b]**
T3 (2.5 ml NNEO's)	51.22[b]	77.45[b]	54.65[a]	22.80[b]	347[c]	219.75[bc]	1.82[a]	33.55[b]	**52.92[cd]**
T4 (5 ml NNEO's)	45.12[c]	68.00[c]	50.05[b]	17.95[c]	371.25[bc]	238.50[b]	1.67[ab]	35.72[b]	**55.65[bc]**
T5 (10 ml NNEO's)	37.27[d]	55.57[e]	39.22[d]	16.35[c]	441.75[a]	289.25[a]	1.27[c]	43.40[a]	**66.12[a]**
±SE	1.05	1.48	1.32	0.61	15.91	9.08	0.11	1.26	**1.27**
P value	0.0001	0.0001	0.0001	0.0001	0.0008	0.0001	0.002	0.0001	**0.0001**

The means of rows a, b,.. e with different superscripts shows a significant difference (P<0.05).

3.7 Breast and thigh fatty acids:

Effect of different treatments on breast and thigh fatty acids profile are presented in Tables (10 and 11), it is clear to note that all added additives significantly decreased the amount of all saturated fatty acids (Lauric, Myristic, Palmitic and stearic) also, total saturated fatty acids as compared to control in breast and thigh muscles. On the other hand, different mono- unsaturated (Palmitoleic and Oleic) was and total mono-unsaturated fatty acids significantly increased by increasing NEEO's level for both breast and thigh meat. The same trend was observed in poly –unsaturated fatty acids (Linoleic, Linolenic, Eicosadienoic acid, Dihomo-γ-linolenic, Arachidonic, Eicosapentaenoic “EPA”, Docosapentaenoic “DPA”, Docosahexaenoic “DHA”) as mentioned in Tables 8 and 9.

This finding agrees with that of Mohamed and Abd El-Wahab (2022), who examined the effects of a blend of garlic, tea tree and pomegranate oils, in normal or Nano emulsion form on rabbit fatty acid fractions at a rate of 7.5 ml/kg diet. They concluded that adding nano-emulsified essential oil lowered total saturated fatty acids while improving mono and polyunsaturated fatty acids. In addition, Abdallah *et al.*, (2022) study on weaned Mountain rabbits proved that adding 7.5 ml/kg diet nano-emulsified essential oil mix (garlic, onion, and lemon essential oil) recorded greater values of mono-unsaturated fatty acids and total poly- unsaturated fatty acids.

Table 10. Effect of different treatments on fractionation of breast fatty acids (mg/100g fat)

Items	T1	T2	T3	T4	T5	±S E	*P value*
Lauric acid (C12:0)	36.00^{a}	30.00^{b}	21.00^{d}	26.00^{c}	19.00^{d}	0.88	0.0001
Myristic (C14:0)	74.00^{a}	36.00^{d}	45.00^{b}	40.00^{c}	27.00^{e}	0.73	0.0001
Palmitic (C16:0)	1269.00^{a}	1062^{d}	1133^{b}	1108^{c}	961^{e}	0.75	0.0001
Stearic (C18:0)	605^{a}	398^{d}	471^{b}	424^{c}	337^{e}	0.77	0.0001
Total Saturated F. As	1984^{a}	1526^{d}	1670^{b}	1598^{c}	1344^{e}	2.18	0.0001
Palmitoleic (C16:1)	311^{e}	457^{c}	425^{d}	481^{b}	574^{a}	1.06	0.0001
Oleic (C18:1)	1282^{e}	1632^{c}	1443^{d}	1695^{b}	1721^{a}	0.71	0.0001
Total Mono-Unsaturated F. As	1593^{e}	2089^{c}	1868^{d}	2176^{b}	2295^{a}	1.29	0.0001
Linoleic (C18:2)	627^{e}	769^{b}	681^{d}	714^{c}	790^{a}	0.68	0.0001
Linolenic (C18:3)	119^{e}	205^{b}	170^{d}	187^{c}	211^{a}	1.06	0.0001
Eicosadienoic acid (C20:2)	17^{d}	37^{a}	25^{c}	32^{b}	39^{a}	1.22	0.0001
Dihomo-γ-linolenic (C20:3)	25^{e}	51^{b}	38^{d}	46^{c}	55^{a}	0.89	0.0001
Arachidonic (C20:4)	130^{e}	178^{b}	162^{d}	169^{c}	193^{a}	0.86	0.0001
Eicosapentaenoic “EPA” (C20:5)	22^{c}	32^{ab}	25^{c}	29^{b}	35^{a}	1.00	0.0001
Docosapentaenoic “DPA” (C22:5)	16^{d}	39^{a}	23^{c}	31^{b}	40^{a}	1.77	0.0001
Docosahexaenoic “DHA” (C22:6)	15^{c}	26^{a}	19^{b}	22^{b}	29^{a}	1.00	0.0001
Total Poly-Unsaturated F. As	971^{e}	1373^{b}	1143^{d}	1230^{c}	1392^{a}	4.09	0.0001

The means of rows a, b,.. e with different superscripts show a significant difference (P<0.05).

T1: control, T2: antibiotics Oxytetracyclinne 50 mg, T3: 2.5 ml NEEO's /kg diet, T4: 5 ml NEEO's /kg diet, T4: 10 ml NEEO's /kg diet.

Table 11. Effect of different treatments on fractionation of thigh fatty acids (mg/100g fat)

Items	T1	T2	T3	T4	T5	±S E	*P value*
Lauric acid (C12:0)	48^{a}	26^{d}	39^{b}	31^{c}	17^{e}	1.02	0.0001
Myristic (C14:0)	91^{a}	35^{d}	64^{b}	50^{c}	29^{e}	1.21	0.0001
Palmitic (C16:0)	1274^{a}	1147^{d}	1228^{b}	1193^{c}	1015^{e}	0.87	0.0001
Stearic (C18:0)	635^{a}	396^{d}	511^{b}	407^{c}	362^{e}	0.82	0.0001
Total Saturated F. As	2084^{a}	1604^{d}	1842^{b}	1681^{c}	1423^{e}	2.19	0.0001
Palmitoleic (C16:1)	310^{e}	476^{c}	435^{d}	519^{b}	588^{a}	0.98	0.0001
Oleic (C18:1)	1251^{e}	1690^{a}	1492^{d}	1584^{c}	1627^{b}	0.84	0.0001
Total Mono-Unsaturated F. As	1561^{e}	2166^{b}	1927^{d}	2103^{c}	2215^{a}	1.26	0.0001
Linoleic (C18:2)	573^{e}	683^{b}	628^{d}	650^{c}	742^{a}	0.97	0.0001
Linolenic (C18:3)	121^{e}	165^{b}	146^{d}	158^{c}	171^{a}	0.91	0.0001
Eicosadienoic acid	14^{d}	34^{b}	21^{c}	32^{b}	39^{a}	0.93	0.0001

(C20:2)							
Dihomo-γ-linolenic (C20:3)	23[e]	51[b]	29[d]	45[c]	57[a]	1.10	0.0001
Arachidonic (C20:4)	130[e]	176[b]	152[d]	169[c]	185[a]	0.88	0.0001
Eicosapentaenoic "EPA" (C20:5)	18[c]	29[a]	24[b]	27[ab]	30[a]	1.02	0.0001
Docosapentaenoic "DPA" (C22:5)	15[c]	40[a]	17[c]	34[b]	38[a]	0.91	0.0001
Docosahexaenoic "DHA" (C22:6)	11[c]	19[b]	14[c]	25[a]	27[a]	1.00	0.0001
Total Poly-Unsaturated F. As	905[e]	1197[b]	1031[d]	1140[c]	1289[a]	2.76	0.0001

The means of rows a, b,.. e with different superscripts show a significant difference (P<0.05).
T1: control, T2: antibiotics Oxytetracyclinne 50 mg, T3: 2.5 ml NEEO's /kg diet, T4: 5 ml NEEO's /kg diet, T4: 10 ml NEEO's /kg diet

3.8 Economic efficiency

Results in Table (12) show that the best relative economic efficiency was for 5 ml NEEO's group (195.55%) followed by 2.5 ml NEEO's group (184.44%) then 10 ml NEEO's group (173.33%) finally antibiotic group (155.55%) compared to control (100%). This result matches up with AL-Ramamneh (2017) who findings that adding onion extract improved the treated chickens feed conversion ratio, weight gain, and feeding efficiency and lowering production costs. And according to research by Ismail *et al.* (2023), feeding weaned rabbits with Cardamom essential oil nano emulsion 400 mg/kg had significant effects on their economic efficiency.in contrast to the control.

Table 12. Economical feed efficiency in different experimental groups:

Items	Economic efficiency						
	LBW (g)	Total revenue/g gain (LE)	Total feed intake / chick (g)	Total feed cost / chick (LE)	Net revenue/ chick (LE)	Economic efficiency (EEf)	Relative EEf%
T1(Control)	1782.50	58.82	3120.25	40.56	18.26	0.45	100
T2 (Oxytetracyclinne 50 mg)	1851.75	61.11	2715.50	35.98	25.13	0.70	155.55
T3 (2.5 ml NNEO's)	2009.00	66.30	2766.75	36.20	30.10	0.83	184.44
T4 (5 ml NNEO's)	1891.50	62.42	2521.75	33.20	29.22	0.88	195.55
T5 (10 ml NNEO's)	1953.50	64.47	2716.00	36.21	28.26	0.78	173.33

Conclusion

The results of adding the nano-emulsified essential oil blend with all tested levels showed a clear improvement in the productive performance of broiler, and the best level of added Nano-emulsion essential oils blend (garlic and onion) is 10 ml NEEO's ml/kg diet which translated as improvement in growth performance, chemical meat quality, mono and poly- unsaturated fatty acids, and finally reflected on economic efficiency.

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مزيج الزيوت المستحلبة النانومترية كبدائل خضراء للمضادات الحيوية على الاداء الانتاجى لدجاج التسمين

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تم تصميم الدراسة الحالية لتقييم تأثير إضافة مستويات مختلفة من مزيج الزيوت الأساسية المستحلبة النامترية على الأداء الانتاجى لدجاج التسمين، وصفات الذبيحة، وهضم العناصر الغذائية والكفاءة الاقتصادية. تم توزيع عدد 200 كتكوت اربور ايكرز غير مجنس بعمر يوم عشوائيا على 5 معاملات متساوية تحتوي كل منها على 40 كتكوت (10 طيور / مكرر). تم تغذية المجموعة الأولى بنظام غذائي قياسي وعملت كمجموعة مقارنه .(T1) المجموعة الثانية تم تغذيتها على عليقة مدعمة بالمضادات الحيوية (أوكسيتتراسيكلين 50 ملجم/كجم عليقة) كمجموعة مقارنه موجبة .(T2) تم تغذية ثلاث مجموعات أخرى بنظام غذائي أساسي مكمل بمزيج من الزيوت الأساسية المستحلبة النانوية (الثوم والبصل) بالمعدلات الاتية (2.5, 5, 10 مل/كجم علف) وتم تصنيفها على أنها T3 و T4و T5على التوالي. واستمرت الدراسة لمدة 35 يوما. سجلت الطيور المغذاة على المعاملة الرابعة أفضل معامل هضم للدهون % EE بينما انخفض معنوياً معامل هضم الالياف الخام CF% بإضافة المعاملات مختلفة مقارنة بمجوعة المقارنه. كان أعلى معدل نمو وزن جسم مكتسب خلال فترة النمو الكلي للطيور التي تم تغذيتها على عليقة 2.5 مل مخلوط زيوت مستحلبة نانومترية ، في حين أن أفضل معدل تحويل غذائي كان للطيور المغذاة على عليقة 5 مللى مخلوط زيوت مستحلبة نانومترية. سجلت الكتاكيت التي تم تغذيتها على 10 مللي مخلوط للزيوت المستحلبة النانومترية أقل عدد من البكتيريا المعوية، وقيم الدهون، وإجمالي الأحماض الدهنية المشبعة في عضلات الصدر والفخذ .تم تحقيق أفضل قيم مضادات الأكسدة والحالة المناعية وكذلك الأحماض الدهنية الأحادية والمتعددة غير المشبعة في الصدر والفخذ في مجموعة المغذاة على 10 مل مخلوط للزيوت المستحلبة النانومترية .