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Influence of Emulsified and Nano-Emulsified Essential Oils Blend on Performance and Meat Characteristics of Weaned Mountain Rabbits

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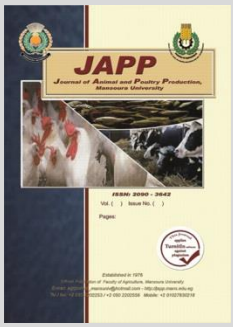

ABSTRACT

The study aimed to evaluate the effects of adding emulsified or nano- emulsified essential oil blend (garlic, pomegranate, tea tree essential oil), at the same level to weaned Mountain rabbits diet, on their growth performance, blood components, meat analysis, meat fatty acids and immunity indices. A total of forty-five weaned Mountain rabbits, were assigned randomly to 3 similar groups. Rabbits in the 1st group (T1, the control) were fed only concentrate feed mixture. The 2nd group (T2) and 3rd group (T3) were fed concentrate feed mixture supplemented with either 0.75 ml/kg diet emulsified, or 0.75 ml/kg diet nano- emulsified essential oil blend, respectively. Results of growth performance revealed that T2 and T3 recoded (P<0.05) higher final live body weight compared with T1, while, T2 and T3 recorded (P<0.05) higher hot carcass weight compared with T1. Regarding meat protein content for the three tested groups T3 recorded (P<0.05) higher value and (P<0.05) the lowest fat content, TVN and TBA compared with T1. Rabbits of T1 found to have (P<0.05) higher triglycerides, cholesterol, urea, creatinine AST and ALT compared with T2 and T3. Immunity values in terms of IgG and IgM were (P<0.05) higher in favor of T3. T3 recorded the best significant (P<0.05) antioxidant in term of GPx and SOD. The control group T1 recoded (P<0.05) the highest significant values of total saturated fatty acids. Opposite trends were realized regarding monounsaturated fatty acids and total poly unsaturated fatty acids where T3 had (P<0.05) higher significant values.

Keywords: Emulsion; Essential Oil; Nano-emulsion; Performance; Rabbit.

INTRODUCTION

Increasing public health concerns and demands for food safety, antibiotic alternative and high-quality white meat has promoted a scientific drive to search about safe and natural alternatives. Herbal plants as well as its essential oils (EOs) have long been used for their therapeutic properties including antimicrobial properties. Application in animal nutrition was neglected, but lately they regained much interest after the banning of antibiotics in animal feed. The most recognized properties of certain plant extracts are their antimicrobial and antioxidant effects, even if the supports of these activities are still partially recognized. Moreover, to its antimicrobial and antioxidants activity, EOs have several biological activities such as hypocholesterolemia, affecting flavor, stimulating the digestion process, antiviral, antimycotic, antiparasitic, properties as well as inhibition of odor and ammonia control.

The EOs are aromatic mixtures, volatile, contain mostly of phenylpropane derivatives and terpenes. it's mainly presence in the plant tissues, linked with plant protection from parasites and bacteria attack Chouhan *et al.*, (2017). The EOs composition may differ depending on vegetative stage, geographical origin of species. Utilization EOs into animal nutrition has promising prospect as health and growth promoter without negative effect on animal, Horky *et al.*, (2019) and Mucha and Witkowska (2021). Plants extracts and their EOs are nowadays largely marketed to be used in animal feeding and are defined as immune and digestive enhancers. EOs are concentrates of aromatic substances and active ingredients; hence their administration should be at well-defined low doses Hengxiao *et al.*, (2018).

Garlic essential oil (Allium sativum L.) consists of terpenoids, saponins, polyphenols, flavone, tannins, quinine, alkaloids, esters, on-volatiles residues and flavonoids. These substances have many useful effects as digestive enzyme enhancer, anti-coccidail, antimicrobial and antioxidants, for better utilization of nutrients to improving digestion, liver function and absorption Li *et al.*, (2012).

Recent studies increased interest in Pomegranate essential oil (PEO) due to their nutritional characteristics and is considered health promoter and defined as a functional food. The PEO has many beneficial compounds that offer antioxidant effects, like sterols, phospho, carotenoids and lipids tocopherols (Verardo *et al.*, 2014). Several biological effects like anti-inflammatory, antioxidant activity, antimicrobial activities, inhibition of platelet aggregation, and have been demonstrated for plant phenolics (He *et al.*, 2011). In recent years, polyphenols have received a great deal of consideration, because of their various biological functions. Phenolic compounds may represent beneficial effects through their free radical scavenging and antioxidant properties, Tannins exist in many plants including pomegranate (*Punica granatum L.*) which are high molecular weight phenolic compounds. Tannins which are high in pomegranate have antimicrobial activity, as well as antioxidant activity (Givi *et al.*, 2019).

Tea tree essential oil (TTEO) (*Melaleuca alternifolia*) is volatile EOs extract from an Australian native plant. used in general for its antimicrobial activities. The TTEO is

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considered as the active component in numerous topical formulations for treat dermal infections. It is extremely available over the counter in North America, Australia and Europe (Thomsen *et al.*, 2011 and Hammer *et al*., 2012).

Nano emulsions are the ultimate useful nanotechnology applications. They are a type of emulsions with droplet sizes ranged from 20 nm to 100 nm. In animal nutrition (Gutiérrez *et al*., 2008). Nano emulsified EOs form had a better effect on increasing of fatty acids (n-3 and n-6). Nano-emulsified EOs had a desirable influence on lowering the biohydrogenation rate of polyunsaturated fatty acids into saturated fatty acids. Supplements of nano emulsified oil tended to be highly influenced than supplements of raw oils (El-Sherbiny *et al*., 2016).

The aim of the current study is to compare and evaluate the effect of supplementing emulsified and nano-emulsified essential oil blend (garlic, pomegranate and tea tree essential oil) to balanced rations of growing rabbits on their growth productive performance, antioxidant status, immune response, carcass traits, meat quality, meat fatty acids and blood biochemistry.

MATERIALS AND METHODS

Formulation of emulsion and nano-emulsion oil

Emulsion was prepared through mixing the 15% oil (5% garlic: 5% pomegranate: 5% tea tree) and surfactant 5.6% (Tween 80) then addition of 79.4% distilled water. The formulation of nano-emulsion was prepared through using magnetic stirrer at 1000 rpm for 10- 20 min at 25 C°. and nano-emulsion formulation was characterized. (Ragavan *et al*., 2017 and Kentish *et al*., 2008).

Characterization of Nano emulsion

Zeta potential

The Nano emulsion surface charge was measured by Laser Doppler electrophoresis, (SZ-100, Horiba Scientific, Kyoto, Japan). diluted samples by deionized water then injected inside a capillary cell to measure the charge at 25 C°, zeta potential values unit in mV. Resulting in apparent zeta potential image (figure 1.).

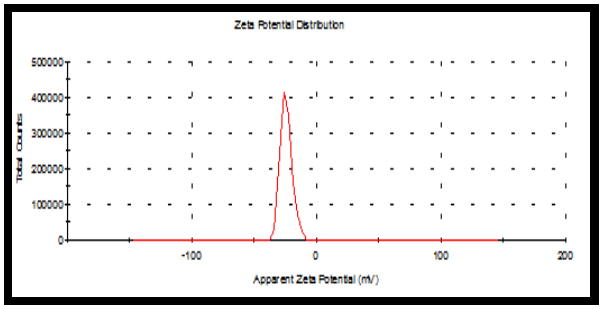

Figure 1. Zeta Potential of Nano- Emulsified essential oil Blend

Transmission electron microscopy (TEM)

The TEM was used to examine the EOs blend nano-emulsion structure and morphology using (FEI-TECNAI G2- 20 TWIN, Netherland). The nano-emulsions were diluted with deionized water at 10 and 100-fold little drops and putted in a carbon film-coated 300 mesh copper grids. The grid could dry 3 hrs. under vacuum and examined by TEM at 80 kV. Resulting in a positive image (Figure 2.).

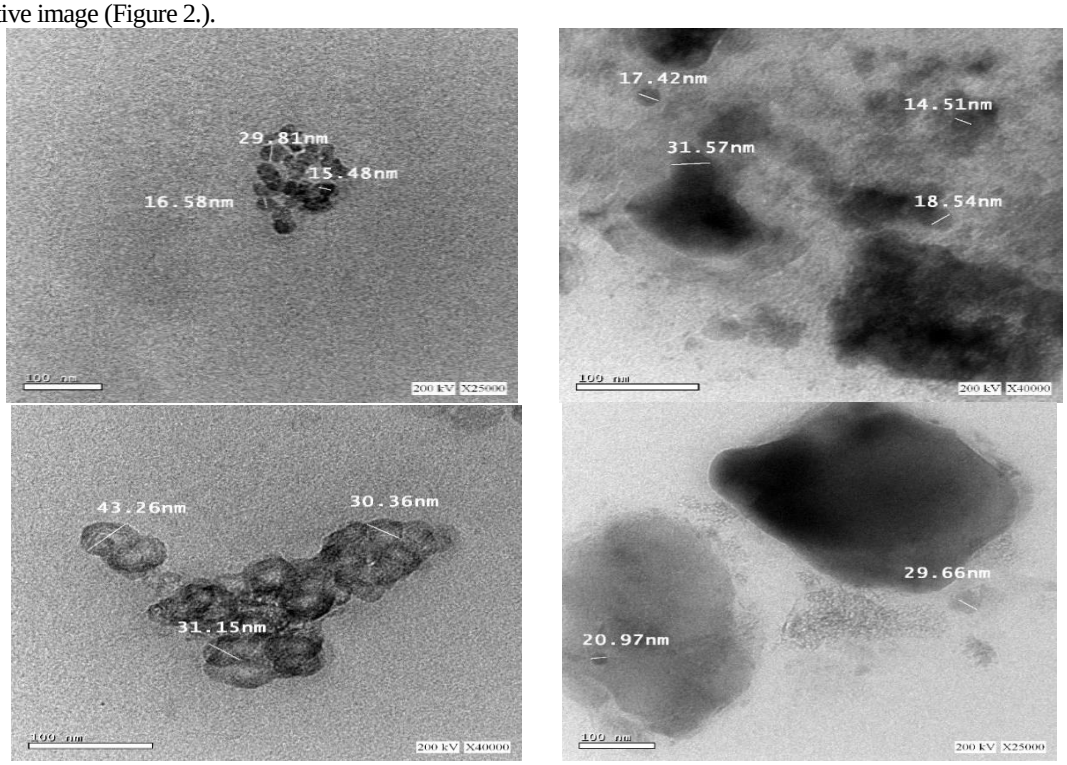

Figure 2. Transmission electron microscopic images of Nano emulsified essential oils blend (garlic, Pomegranate and Tea tree essential oil).

Animals and Diets

This study was conducted at the rabbit experimental farm, Animal Production Department, Fac. of Agri., Benha University. Feed, meat and blood samples analyses were conducted at food analysis center, Fac. of Veterinary Medicine, Benha University, Egypt. Nano emulsion oils preparation and characterization were conducted at Nanotechnology research center, Egyptian Petroleum Research Institute, Nasr City, Cairo, Egypt. Forty-five males weaned Mountain rabbits at 1±0.5 kg were randomly

assigned to three groups (n=15). Weaned Mountain rabbits were weighed biweekly to determine their body weight. The experiment work lasted for 75 days. Experimental group, T1: Control group fed ration without any supplement, while T2: fed the control ration + 0.75 ml mixture of emulsified (Garlic, Pomegranate and Tea tree) oils blend per kg diet, and T3: fed on control ration + 0.75 ml Nano-emulsified (Garlic, Pomegranate and Tea tree) oils blend per animal. Ingredient composition and calculated proximate chemical analysis of a commercial diet presented in (Table 1). Each group's rations were weighed and administered 2 times a day to the animal at 7 AM and 7 PM. The offered feeds were determined to cover the weaned Mountain rabbit's nutrient requirements according to the Ministerial decree of Ministry of Agriculture and Land Reclamation (1996).

Table 1. Commercial diet composition and calculated chemical analysis

Ingredients	**(%)**
Yellow corn	18
Wheat bran	11
Soybean meal (44%CP)	25
Barley	12
Clover hay	30
Limestone	1.50
Di- Calcium phosphate	2
NaCl	0.50
Total	100
Calculated analyses	
DE (kcal/kg)	1465.44
CP%	20.42
CF%	14.05
EE %	1.73
Ca %	1.38
Ph%	0.46
Lys. %	1.16
Meth.+ Cyst. %	0.58

DE: Digestible energy(kcal/kg), CP: Crude protein%, CF: Crude fiber%, EE: Ether extract%, Ca: Calcium%, Lys.: Lysine%

Carcass traits:

Three random rabbits were taken from each group and fasted for 12 hours at the end of the trial period, weighed individually and sacrificed. After complete bleeding, belt and viscera were removed and then carcass and giblet (liver, heart and kidney) were weighed. The dressing percentage was calculated according to Steven *et al*., (1996).

Chemical composition determination of rabbit's meat:

The examined samples of rabbit meat were analyzed for their contents of moisture, protein, fat, and ash according to AOAC (2005). Determination of meat pH was done according to Pearson (2006), while Total Volatile Nitrogen (TVN) determined according to Egyptian Organization for Standardization (ES: 63/9/2006)

TVN/l00g= (mls H2 So4 n0.1 for sample – ml H2 So4 n0.1 for Blank) x 14

The determination of Thiobarbituric Acid Number (TBA) depended on testing malonaldehyde (MDA) as a product of lipid peroxidation TBA number or values. Absorbance of sample was measured using Spectrophotometer (UNICAM969AA Spectronic, (USA) under wavelength 538, Egyptian Organization for Standardization (ES:63/10/ 2006).

TBA value= absorbance of sample x 7.8 (malonaldehyde (mg) /Kg)

Antioxidant capacities of rabbit's meat:

Glutathione Peroxidase (GPx) as an Antioxidant capacities was determined commercially by GPx kits (Randox, Crumlin, UK) and expressed as unit/mg protein according to Fang *et al*., (2011), The activities of superoxide dismutase (SOD) and catalase (CAT) were analyzed according to the methods described by Wang *et al*., (2011). Malondialdehyde (MDA) rabbit serum was estimated and expressed by U/mg protein according to Wang *et al.,* (2011).

Fractionation of fatty acid

Fatty acids (FAs) were determined in rabbit's meat by Gas Chromatography technique (GC) according to Aura *et al*., (1995). Extraction of fat was according to AOAC (2000). The extracted of FAs were diluted in 0.5-1.0 ml anhydrous diethyl ether and methylated by adding diazomethane solution drop by drop until the yellow color was maintained (Vogol 1975). The GC analysis was performed by Hewlett Packard gas chromatography (5890 series) with flame ionization detector.

Blood parameters:

Blood samples were taken from jugular vein of 5 rabbits of each group. Blood samples (5 ml) were taken 4 hrs. after feeding of each rabbit into a clean dry tube without adding anticoagulants. Blood samples were centrifuged at 3000 pm for 30 min to get blood serum. Serum was separated into 2-ml clean dried Eppendorf tubes and frozen at −20°C for later analysis. Albumin was determined by the method of Doumas *et al*., (1971), Globulin was calculated by subtracting the albumin from total protein. The estimation of total cholesterol in rabbit serum was expressed as mg/dL and measured according to Roschlau (1974). Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) results were expressed as IU/L and were determined according to Reitman and Frankel (1975). Creatinine expressed as mg/dL was applied according to the technique recommended by Julian (2000) by using the available commercial kits provided by Biomerieux, France). Triglycerides rabbit's serum was determined according to Fossati and Prencipe (1982). The blood urea nitrogen was determined by enzymatic colorimetric according to Patton and Cronch (1979). The immunoglobulin G (IgG) and M (IgM) levels were determined in the prepared rabbit serum using commercial bio diagnostic kits provided from Bio diagnostic Company (Giza, Egypt) and a spectrophotometer (Shimadzu, Japan) following the instruction directions.

Statistical analysis

Each parameter was analyzed using SAS (2013). According to the design of experiment, the one-way analysis was followed. Then, Duncan's multiple range test (Duncan, 1955) was applied to compare the significance among mean values.

The mathematical model was: $Yij = \mu + Ti + eij$

Where: μ, Ti, Yij and eij refer to overall mean, effect of treatment, Individual observation and random error, respectively.

RESULTS AND DISSCUSION

Productive performance:

As shown in Table 2, results of productive performance of weaned rabbits as affected by supplementation with emulsified essential oil blend (EEOs) and nano-emulsified essential oil (NEEOs) blend revealed no significant differences among the three tested groups in the average initial LBW, while T2 and T3 recorded significantly ($P<0.05$) higher final LBW compared with the control (T1). It was also noticed that T3 achieved significantly ($P<0.05$) higher average daily BWG compared with T1. In the

meantime, differences among T3 and T2 were not significant as well as between T2 and T1. The same trend was observed in FCR where T3 recorded the best FCR (P<0.05) compared with T2 and T1. The EOs is often has the potential to improve the palatability and flavor of feed, so rising voluntary feed intake, which reflected into improving the weight gain. The positive results for most of productive performance parameters in favor of groups fed ration supplemented with emulsified blend of essential oils and nano emulsified essential oil (garlic, pomegranate, tea tree essential oil), could be attributed to the fact that emulsification and nano processes have altered the composition of the treated materials post application. In general, emulsification has the effect to increase the surface size of such material which allow enzymatic action during digestion process to work more efficiently in transforming raw materials into simple metabolites compared to untreated ones.

Nano-emulsion is an isotropic blend, a mixture of surfactant and oil which spontaneously forms fine emulsions of oil in water (Mason *et al*., 2006). Nano process acts to minimize sizes of the raw material particles to very tiny particles which allow efficient digestion process and higher and easier penetration of those tiny particles within different part of the digestive system.

The nanoparticle commonly being used to protect the essential oil from oxidation. Nanoparticle size of essential oils can reduce the possibility of degradation, increase stability, function, increase the shelf life of the products and increasing the free of the essential oil bioactive molecules (Wang *et al.*, 2009). Rong *et al*., (2012) reported that nanoparticles can be made to be nanocarriers which, promote their activity via providing various diffusion properties that make them pass through the biological membranes because of the nanoscale particles droplets size.

Therefore, the two techniques could be basically the cause of better use of feed materials and nutrients by the animal in T2 and T3 compared with controlled ones.

The EOs have been greatly used for medicinal usage, fungicidal, bactericidal, antiparasitic, virucidal, insecticidal, mostly in pharmaceutical, cosmetic, sanitary and agricultural and food industries. Therefore, these functions could be of physiological benefits that help animal to control pathogenic bacteria along its gut to promoting growth and preventing diseases (Pedro *et al.*, (2013), Bakkali *et al*., (2008) and Baskara *et al*., (2020)). Landoni and Albarellos (2015) reported that EO's seem to get rid of harmful microbes, stimulate useful microorganisms like Lactobacillus *spp.* and stimulate the enzymes activity, and generally improved feed conversion ratio.

The author also concluded that EO's include many components that have the ability to manipulate positively rumen fermentation and gut microbiota, prevent tissue oxidation and inhibit growth of pathogenic bacterial, which in turn improve growth performance and products' quality of farm animals. Opposite to the results of our study, Simitzis (2017) reported that no significant differences were detected in feed intake, live body wight, body weight gain and feed conversion ratio in White New Zealand rabbits fed supplemented ration with garlic essential oil over the starter and finisher experimental periods related to garlic addition. However, there is numerical increased in life body weight, body weight gain and feed conversion ratio of rabbits fed diet supplemented with garlic essential oil during the experiment period (7-13 Week).

Table 2. Effect of emulsified, or nano-emulsified essential oils blend on productive performance of weaned Mountain rabbits.

Items	Experimental rations			
	T1	T2	T3	±S E
Initial BW, kg	1.05	1.04	0.99	0.04
Final BW, kg	1.91^{b}	1.97^{a}	2.01^{a}	0.05
DBW gain, g	11.47^{b}	12.41^{ab}	13.60^{a}	0.56
Average daily feed intake (on DM basis) g/h/d	85	85	85	-----
Feed conversion ratio	7.41^{a}	6.84^{ab}	6.25^{b}	0.31

T1, T2 and T3 refer to (control diet), control diet supplemented with 0.75 ml EEOs/ kg diet and 0.75 ml NEEOs/kg diet, respectively. a and b letter showed the significant variation (P<0.05) among mean values within each experimental rations.

Carcass traits and meat chemical analysis

Results of carcass traits of the three tested groups (T1, T2 and T3) are shown in Table 3. Statistical analyses showed thatT2 and T3 recorded higher significant (P<0.05) hot carcass weight compared with the control (T1). No significant difference was observed among three tested groups in dressing percentage. Regarding carcass cut weight, T2 and T3 achieved the highest (P<0.05) round and belt weight compared with the control (T1), while, differences were not significant among the three tested groups in lion and shoulder weight. On the other hand, T 2 recoded higher (P<0.05) rack weight compared with T1 and T3.

As shown in Table 4, it was observed that meat moisture content was found to be (P<0.05) higher in T1 compared with T2 and T3 respectively. The opposite result was observed regarding to protein content for the three tested groups where T3 recorded (P<0.05) higher value, in a decreasing order, compared with T2 and T1 respectively. Regarding fat content, T3 recorded (P<0.05) the lowest fat content followed in a deceasing order by T2 and T1. Insignificant (P<0.05) differences were observed among the three tested groups in values of ash and pH. Values of TVN and TBA as affected by EEOs and NEEOs showed that T1 recorded higher (P<0.05) values compared with T3 and T2 respectively. Similar to our results, Cardinali *et al*., (2015) reported that rabbit feed supplemented with oregano extract (0.2 mL/100 g) or oregano and rosemary extracts (0.1 mL/100 g, each) exhibited the highest final live body weight, feed conversion ratio and carcass weight. Mattioli *et al*., (2017) fed New Zealand rabbits diets containing oregano extract, prebiotic and vitamin E. showed reduction in lipid oxidative stress for all the experimental groups in the rabbit's loin. All diets positively increased PUFAs. Rabbit fed diets containing plant extract produced meat of higher quality rich in ω6/ω3. Moreover, plant extracts and EOs are also reported to increase redox balance in various organs (Zeng *et al*., 2015 and Mattioli *et al*., 2017). Simitzis (2017) reported insignificant improvement of carcass trait and meat quality of New Zealand rabbits fed diet supplemented with garlic oil. These results are also in agreement with results obtained by Onibi *et al*., (2009) and Raeesi *et al*., (2010).

Table 3. Effect of emulsified, or nano-emulsified essential oils blend on rabbit carcass.

Items	Experimental rations			
	T1	T2	T3	± SE
Live Condition Score	3.66[b]	4.33[a]	4.34[a]	0.70
Final live weight (kg)	1.956[c]	2.165[a]	2.081[b]	0.016
Hot Carcass Weight (kg)	0.916[b]	1.028[a]	0.988[a]	0.013
Dressing Percent (%)	61.83	62.98	63.38	0.71
Carcass offal's weight (g)				
Legs weight	0.58[b]	0.73[a]	0.70[a]	0.453
Hand weight	0.120[b]	0.133[a]	0.123[b]	0.027
Heart weight	0.004	0.006	0.005	0.006
Digestion tract content	0.343	0.426	0.430	0.038
Lungs and trachea	0.018[b]	0.061[a]	0.013[b]	0.025
Liver weight	0.866	0.900	0.950	0.20
Kidneys weight	0.153[a]	0.163	0.153	0.010
Carcass cuts weight (g)				
Round weight	0.360[b]	0.420[a]	0.416[a]	0.016
Loin weight	0.236	0.219	0.240	0.007
Rack weight	0.171[b]	0.233[a]	0.185[b]	0.011
Shoulder weight	0.146	0.161	0.162	0.004
Belt weight	0.250[b]	0.270[a]	0.265[a]	0.008

T1, T2 and T3 refer to (control diet), control diet supplemented with 0.75 ml EEOs/ kg diet and 0.75 ml NEEOs/kg diet, respectively. a and b letter showed the significant variation (P<0.05) among mean values within each experimental rations.

Table 4. Effect of emulsified, or nano-emulsified essential oils blend on rabbit meat chemical analysis of weaned mountain rabbits.

Items	Experimental rations			
	T1	T2	T3	±S E
Moisture %	74.26[a]	73.73[ab]	72.96[b]	0.27
Protein %	19.90[b]	20.96[ab]	22.00[a]	0.31
Fat %	2.60[a]	2.16[ab]	2.03[b]	0.14
Ash %	1.80	1.90	2.06	0.13
pH	5.77	5.70	5.65	0.03
TVN mg%	5.10[a]	3.60[b]	2.63[b]	0.30
TBA mg/Kg	0.31[a]	0.22[ab]	0.17[b]	0.03

T1, T2 and T3 refer to (control diet), control diet supplemented with 0.75 ml EEOs/ kg diet and 0.75 ml NEEOs/kg diet, respectively. a and b letter showed the significant variation (P<0.05) among mean values within each experimental rations.

Rabbit meat antioxidant analysis:

As shown in Table 5. T3 recorded significantly (P<0.05) the best antioxidant effect on rabbit meat in term of GPx (U/mg protein) and SOD (U/mg protein) compared with T1, however differences between T3 and T2 and between T2 and T1 were not (P<0.05) significant. Regarding CAT (U/mg protein) T3 and T2 recorded higher values compared with T1, while MDA parameter showed insignificant differences among three tested groups. Animals fed EEOs and NEEOs exhibit significantly (P<0.05) higher antioxidant activities compared with the control. In general, free radicals' production and oxidation of lipids are normally processes that causes destroying cell membrane structure and cell organelles loses its function due to the disturb happen in cell membrane transporting processes, especially phospholipids in cell membranes which are the most sensitive materials to be damage by oxidation that is negatively correlated with the proportion of unsaturated fatty acids. One of the functions of polyunsaturated fatty acids is to maintain of physiologically cell membrane properties which include permeability and fluidity. The pre-oxy radicals react with polyunsaturated fatty acids and produce hydroperoxides, which form the volatile non-radical aromatic compounds (alkanes, conjugated dienes, aldehydes, etc.) which, negatively influence animal products quality, by causing decrease of nutritive value and limiting meat shelf-life El-Gogary (2018). He also reported that supplementing animal diet with garlic oil led to stop the production of free radicals' in rabbit's organs and its products.

Table 5. Effect of emulsified, or nano-emulsified essential oils blend on rabbit meat antioxidant analysis of weaned Mountain rabbits.

Items	Experimental rations			
	T1	T2	T3	±S E
GPx (U/mg protein)	65.00[b]	69.20[ab]	71.83[a]	1.58
SOD (U/mg protein)	322.46[b]	334.90[ab]	345.50[a]	4.65
CAT (U/mg protein)	18.36[b]	20.03[a]	20.70[a]	0.90
MDA (nmol/mL)	1.72	1.51	1.59	0.07

T1, T2 and T3 refer to (control diet), control diet supplemented with 0.75 ml EEOs/ kg diet and 0.75 ml NEEOs/kg diet, respectively. a and b letter showed the significant variation (P<0.05) among mean values within each experimental rations.

The EOs are great sources of natural antioxidants, like phenolic compounds distinguished of chemical structure could neutralize free radicals and highly redox properties (Pisoschi and Pop 2015 and Rice-Evans *et al.*, 1995). Supplementing diets with EO's is a simple and suitable strategy to introduce natural antioxidants substance into phospholipid cell membranes, where potently inhibit the oxidative reactions by free radical's formation, through scavenging them, or by promoting their decomposition at their localized sites and appears as a more active way of slow down the lipid oxidation of animal products compared to post slaughter addition (Zheng and Wang 2001 and Govaris *et al*., 2004). Feeding broilers with 150 mg/kg ginger essential oil led to decrease MDA concentrations and increase total super oxide dismutase activity in liver compared with a control group. Also, supplemented diet with vitamin E, ginger root powder or its essential oil led to decrease serum MDA concentrations and increase serum total antioxidant capacity compared with the control group (Decker and Park 2010).

Rabbit's meat Fatty acids:

Results of fatty fractionation as presented in Table 6. Revealed that the control (T1) recoded the highest significant (P<0.05) values of total saturated fatty acids (Lauric acid (C12:0), Myristic (C14:0), Palmitic (C16:0), Stearic (C18:0)) compared with T3 and T2 respectively. Opposite results were realized regarding to monounsaturated fatty acids (Palmitoleic (C16:1) and Oleic (C18:1)) where T2 and T3 recorded (P<0.05) higher significant values compared with T1. Regarding results of total poly unsaturated fatty acids (Docosahexaenoic "DHA" (C22:6), Linolenic (C18:3), Eicosadienoic acid (C20:2), Dihomo-γ-linolenic (C20:3), Arachidonic (C20:4), Eicosapentaenoic "EPA" (C20:5), Docosapentaenoic "DPA" (C22:5), Linoleic (C18:2)) T3 and T2 found to have (P<0.05) higher significant values compared with T1.

In agreement with our results, Szymczyk and Szczurek (2016) reported in a study using pomegranate seed oil (PSO) and linseed oil (LO) at levels: 0.0, 0.5, 1.0, 1.5% and two levels of LO per PSO level: 0.0 and 2.0% that the feed-to-gain ratio in broilers fed PSO diets was significantly improved. Increasing dietary supplementing ratio of PSO caused a gradual increase in the deposition of conjugated linoleic acid isomers (CLA) in breast meat while breast lipids saturated fatty acids did not influence. Meanwhile, polyunsaturated fatty acids (PUFA) proportions was significantly increased, while, 2% LO significantly increased total PUFA and improved tissue n-6/n-3 ratio.

Table 6. Fractionation of meat fatty acids of weaned mountain rabbits of the experimental groups (mg/100g).

Items	T1	T2	T3	±S E
Lauric acid (C12:0)	39.66^{a}	29.66^{b}	25.66^{c}	0.57
Myristic (C14:0)	80.00^{a}	70.00^{b}	63.33^{c}	0.69
Palmitic (C16:0)	1267.67^{a}	1148.67^{b}	1046.33^{c}	1.58
Stearic (C18:0)	568^{a}	468.67^{c}	519.67^{b}	2.55
Total Saturated F. As	1995.5^{a}	1717^{b}	1651.67^{c}	14.41
Palmitoleic (C16:1)	338.67^{c}	371^{b}	393^{a}	3.62
Oleic (C18:1)	1492.33^{c}	1546.67^{b}	1572.67^{a}	4.65
Total Mono-Unsaturated F. As	1831.33^{c}	1965.67^{a}	1917.67^{b}	9.55
Linoleic (C18:2)	617.33^{c}	663.33^{b}	687.00^{a}	4.67
Linolenic (C18:3)	131.33^{c}	138^{b}	157^{a}	1.71
Eicosadienoic acid (C20:2)	13.33^{b}	16.66^{a}	15.33^{a}	0.47
Dihomo-γ-linolenic (C20:3)	21.33^{b}	23.00^{ab}	24.33^{a}	0.63
Arachidonic (C20:4)	131.00^{c}	137.66^{b}	157.00^{a}	0.96
Eicosapentaenoic "EPA" (C20:5)	11.00^{c}	12.33^{b}	13.66^{a}	0.27
Docosapentaenoic "DPA" (C22:5)	20.00^{b}	21.66^{b}	24.33^{a}	0.54
Docosahexaenoic "DHA" (C22:6)	16.66^{b}	18.00^{ab}	18.66^{a}	0.43
Total Poly-Unsaturated F. As	962.0^{c}	1032.67^{b}	1098.67^{a}	2.27

T1, T2 and T3 refer to (control diet), control diet supplemented with 0.75 ml EEOs/ kg diet and 0.75 ml NEEOs/kg diet, respectively. a, b and c letter showed the significant variation (P<0.05) among mean values within each experimental rations.

Blood analyses:

Results of blood analyses are shown in Table 7. Statistical evaluation revealed that T 3 recorded (P<0.05) the highest albumin values followed in a decreasing order by T2 and T1 respectively. No significant differences were found among the three tested groups in globulin values. The control (T1) was found to have (P<0.05) higher triglycerides compared with T2 and T3 respectively. Similar trends were observed regarding values of cholesterol, urea creatinine AST, ALT. On the other hand, immunity values in term of IgG and IgM found to be (P<0.05) higher in favor of T3 and T2 compared with the control (T1).

In agreement with our obtained findings, supplementing EOs under practical conditions of large-scale animal production have shown excellent responses compared with experiment conducted under ideal condition and higher level of hygiene (Franz *et al*., 2010). This may be due to decrease the pathogen load in the intestinal tract and enhanced overall immune status. Similar to our results, Walter and Bilkei (2004) reported that supplementing EO's has been found to enhance the piglet's immunity after weaning, as showed by an increase in phagocytosis rate, lymphocyte proliferation rate, C3, C4, IgG, IgM and IgA serum levels. Also, they found that pigs fed a diet supplemented with 3 g/kg oregano essential oil increased the proportions of MHC class II antigens, non-T/non-B cells and CD4:CD8 in peripheral blood lymphocytes compared with control diet pig group. The results related to supplementing rabbits' diet with 0.5 g/kg garlic essential oil led to enhance rabbit's humoral immune response, which reflected into in higher significant increases in immunoglobulin. But, the high dose of garlic oil (0.75 g/kg) reduced the immunity response which could be due to the toxic effect of an overdose (Alagawany *et al*., 2016, El-Gogary 2018). El-Gogary (2018) found significant increase in TAC and significant decrease in MDA in the blood as affected by supplanting rabbit's diet with essential oil.

Table 7. Effect of emulsified, or nano-emulsified essential oils blend on rabbit blood Serum analyses of weaned mountain rabbits.

Items	Experimental rations			
	T1	T2	T3	±S E
Albumin (g/dl)	3.33^{b}	3.73^{ab}	3.93^{a}	0.15
Globulin (g/dl)	1.53	1.66	1.70	0.09
Triglyceride (mg/dl)	55.73^{a}	53.83^{b}	52.56^{b}	0.42
Cholesterol (mg/dl)	63.16^{a}	61.86^{ab}	61.13^{b}	0.39
Urea (mg/dl)	26.86^{a}	24.46^{b}	23.20^{b}	0.38
Creatinine (mg/dl)	0.92^{a}	0.85^{ab}	0.79^{b}	0.02
AST (U/L)	33.60^{a}	31.16^{b}	29.86^{b}	0.53
ALT (U/L)	21.16^{a}	19.03^{b}	17.4^{b}	0.53
IgG (mg/dl)	39.20^{b}	43.93^{a}	45.40^{a}	0.50
IgM (mg/dl)	57.76^{b}	64.06^{a}	65.66^{a}	0.56

T1, T2 and T3 refer to (control diet), control diet supplemented with 0.75 ml EEOs/ kg diet and 0.75 ml NEEOs/kg diet, respectively. a and b letter showed the significant variation (P<0.05) among mean values within each experimental rations.

CONCLUSION

Nano emulsified essential oil blend may be considered promising natural substances to improve nutrient absorption and contributing to body weight gain, feed conversion ratio, improving blood immunoglobulin levels, increased antioxidant activities and production of healthy meat riches in total proportion of mono and poly unsaturated fatty acid. Overall, these results could support the recommendation of supplementing rabbit's diet with NEEOs (garlic, pomegranate, tea tree essential oil) blend at 0.75 ml NEEOs /kg diet and raising awareness of application of nanotechnology in animal nutrition at animal farm level.

REFERENCIES

AOAC (2000). Association of Official Analytical Chemists (13th Ed.). w. Horwitz. w, (Editor), Academic press, Washington, D.C., USA.

AOAC (2005). "Association of Official Analytical Chemists" Official Methods of Analysis. 14th Ed., Horwitz.w; D. (Editor), Academic Press, Washington D.C, USA.

Alagawany M., E..A. Ashour and F.M. Reda (2016). Effect of dietary supplementation of garlic (Allium sativum) and turmeric (Curcuma longa) on growth performance, carcass traits, blood profile and oxidative status in growing rabbits. Annals of Animal Science. 16(2): 1-17.

Aura A.P.; A. Forssell; Mustranta and K. Poutanen (1995). Transesterification of soy lecithin by lipase and phospholipase, Journal of Amer. Oil Chem. Soc., 72: 1375-1379

Bakkali F.; S. Averbeck; D. Averbeck and M. Idaomar (2008). Biological effects of essential oils – a review. Food Chem. Toxicol. 46: 446–475. doi: 10.1016/j.fct.2007. 09. 106

Baskara A.; B. Ariyadi; N. Dono; R. Martien and Z. Zuprizal (2020). The Potential Use of Essential Oil Nanoemulsion as a Novel Alternative to Antibiotics in Poultry Production-A Review, 10: 203-212.

Cardinali R.; M. Cullere; A. Bosco; Dr. Mugnai; S. Ruggeri; S. Mattioli; C. Castellini; M. Trabalza and A. Dalle Zotte (2015). Oregano, rosemary and vitamin E dietary supplementation in growing rabbits: Effect on growth performance, carcass traits, bone development and meat chemical composition. Livestock Science. 175:83-89

Chouhan S.; K. Sharma and S. Guleria (2017). Antimicrobial Activity of Some Essential Oils-Present Status and Future Perspectives. Medicines (Basel). 4(3):58; https://doi:10.3390/medicines4030058.

Decker E.A. and Y. Park (2010). Healthier meat products as functional foods. Meat Sci. 86: 49–55.

Doumas, B.T.; W.A. Watson and H.S. Bigges (1971). Albumin standard and measurement of serum albumin with bromocresol green. Clin. Chem. Acta. 31:87-96.

Duncan D.B. (1955). Multiple Range and Multiple F Test. Biometrics. 11:1-5.

Egyptian Organization for Standardization (ES) (2006). Methods of analysis and testing for meat. Part 10: determination of thiobarbituric acid (TBA). ES: 63/10.

Egyptian Organization for Standardization (ES) (2006). Methods of analysis and testing for meat. Part 9: determination of total volatile nitrogen (TVN). ES:63/9.

El-Gogary M.R., A.M. Mansour and E.A. El-Said (2018). Blood biochemical and immunological responses to garlic oil administration in growing rabbit's diet. Journal of Agricultural Science. 10 (1): 217-224.

El-Sherbiny M.; C. Adam; E. Pers-Kamczyc; J. Szczechowiak-Piglas; D. Kowalczyk and M. Szumacher-Strabel (2016). Short communication: A nanoemulsified form of oil blends positively affects the fatty acid proportion in ruminal batch cultures. Journal of Dairy Science. 99:399–407.

Fang Y.J.; C.H. Chiu and Y.Y. Chang (2011). Taurine ameliorates alcoholic steatohepatitis via enhancing self-antioxidant capacity and alcohol metabolism. Food Research International. 44: 3105–3110.

Fossati P. and L. Prencipe (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clinical Chemistry. 28: 2077-2080.

Franz C.; K. Baser and W. Windisch (2010). Essential oils and aromatic plants in animal feeding–a European perspective. A review. Flavour Frag J. 25:327–340.

Givi F.; M. Gholami and A. Massah (2019). Application of pomegranate peel extract and essential oil as a safe botanical preservative for the control of postharvest decay caused by Penicillium italicum and Penicillium digitatum on "Satsuma" mandarin. Journal of Food Safety. 39: 1–11.

Govaris A.; N. Botsoglou; G. Papageorgiou; E. Botsoglou and I. Ambrosiadis (2004). Dietary versus post-mortem use of oregano oil and/or α-tocopherol in turkeys to inhibit development of lipid oxidation in meat during refrigerated storage. Int. J. Food Sci. Nutr. 55: 115–123.

Gutiérrez J.; C. González; A. Maestro; I. Sole; C.M. Pey and J. Nolla (2008). Nano-emulsions: New applications and optimization of their preparation. Current Opinion In Colloid & Interface Science. 13:245-251.

Hammer K.A.; C.F. Carson; T.V. Riley and K.A. Hammer (2012). Effects of Melaleuca alternifolia (tea tree) essential oil and the major monoterpene component terpinen-4-ol on the development of single- and multistep antibiotic resistance and antimicrobial susceptibility. Antimicrob Agents Chemother. 56(2): 909-915.

He L.; H. Xu; X. Liu; W. He; F. Yuan; Z. Hou and Y. Gao (2011). Identification of phenolic compounds from pomegranate (Punica granatum L.) seed residues and investigation into their antioxidant capacities by HPLC–ABTS+ assay. Food Res. Int. 44: 1161–1167.

Hengxiao Z.; L. Hong; W. Shikui and W. Jinlong (2018). Anna-Maria Kluenter, Potential of essential oils for poultry and pigs, Animal Nutrition. 4(2): 179-186.

Horky P.; S. Skalickova; K. Smerkova and J. Skladanka (2019). Essential Oils as a Feed Additives: Pharmacokinetics and Potential Toxicity in Monogastric Animals. Animals (Basel). 9(6), 352; https://doi:10.3390/ani9060352.

Julian (2000) Laboratory Procedure Manual, Biochemistry Profile, Serum. Hitachi Model., 917 Multichannel Analyzer. Coulston Foundation, Alamogordo, New Mexico, Mexico.

Kentish S.; T. Wooster; M. Ashokkumar; S. Balachandran; R. Mawson and L. Simons (2008). the use of ultrasonic for nanoemulsion preparation. Innovative Food Science & Emerging Technologies., 9(2):170–175.

Landoni M.F. and G. Albarellos (2015). The use of antimicrobial agents in broiler chickens. Vet. J., 205: 21-27.

Li S.Y.; Y.J. Ru; M. Liu; B. Xu; A. Péron and X.G. Shi (2012). The effect of essential oils on performance, immunity and gut microbial population in weaner pigs. Livest. Sci. 145: 119–123.

Mason T.G.; S.M. Graves; J.N. Wilking and M.Y. Lin (2006). Extreme emulsification: Formation and structure of nanoemulsions Condens. Matter Phys. 9: 193-199.

Mattioli S.; R. Cardinali; M. Balzano; D. Pacetti; C. Castellini; A. Bosco and N. Frega (2017). Influence of Dietary Supplementation with Prebiotic, Oregano Extract, and Vitamin E on Fatty Acid Profile and Oxidative Status of Rabbit Meat. Journal of Food Quality. 1-9.

Ministerial decree of Ministry of Agriculture and Land Reclamation (1996).. The standard properties for ingredients, feed additives and feed manufactured for animal and poultry. El-Wakaee El-Masria, Amirria Press Cairo, Egypt. 192:95. Available at: https://www.ecolex.org / details / legislation / decree-no-22-of-1996-of-the-ministry - of – agriculture – on – veterinary - products-lex-faoc012988/

Mucha W. and D. Witkowska (2021) The Applicability of Essential Oils in Different Stages of Production of Animal-Based Foods. Molecules. 26(13): 3798; https://doi.org/10.3390/molecules26133798

Onibi G.E.; O.E. Adebisi; A.N. Fajemisin and A.V. Adetunji (2009). Response of broiler chickens in terms of performance and meat quality to garlic (Allium sativum) supplementation. Afr. J. Agric. Res., 4:511-517.

Patton C.J. and S.R. Cronch (1979). Spectrophotometic and Kkimetics investigation of the Berthelat reaction for the determination of ammonia. Anal.Chemi., 18:59- 69.

Pearson D. (2006). Chemical Analysis of Foods. 11th Ed, Publishing Co., Churchill Livingstone, Edinburgh, London, United Kingdom.

Pedro A.S.; I.E. Santo; C.V. Silva ; C. Detoni; E. Albuquerque; E. Politécnica and R. Novis (2013). The use of nanotechnology as an approach for essential oil-based formulations with antimicrobial activity. science, technology and education 1364-1374

Pisoschi, A.M. and A. Pop (2015) The role of antioxidants in the chemistry of oxidative stress: A review. Eur. J. Med. Chem. 97: 55–74.

Raeesi M.; S.A. Hoeini-Aliabad; A. Roofchaee; A. ZareShahneh and S. Pirali (2010). Effect of periodically use of garlic (Allium sativum) power on performance and carcass characteristics in broiler chickens. World Academy of Science, Engineering and Technology. 68:1213-1219.

Ragavan G.; Y. Muralidaran; B. Sridharan; R. Nachiappa Ganesh and P. Viswanathan (2017). Evaluation of garlic oil in nano-emulsified form: optimization and its efficacy in high-fat diet induced dyslipidemia in Wistar rats. Food Chem Toxicol. 105: 203–213.

Reitman S. and Frankel (1975). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Animal j. Clinical pathology, 28:56-63.

Rice-Evans C.A. (1995). Miller, N.J.; Bolwell, P.G.; Bramley, P.M.; Pridham, J.B. The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radic. Res. 22: 375–383.

Rolschlau, P. Klin. (1974). Biochem., 12, 226.

Rong L.; X. Shiqi; S. Charles; L. Yue; Z. Fang and H. Qingrong (2012). Physical and Antimicrobial Properties of Peppermint Oil Nanoemulsions. Journal of agricultural and food chemistry. J. Agric. Food Chem., 60: 7548−7555.

SAS (2013). SAS Procedure Users Guide. "version 6.12 Ed". SAS Institute Inc., Cary, NC, USA.

Simitzis P.E. (2017). Enrichment of Animal Diets with Essential Oils—A Great Perspective on Improving Animal Performance and Quality Characteristics of the Derived Products. Medicines, 4:35. https://doi.org/10.3390/ medicines 4020035

Steven W.D.; W.D. Hohenboken; P.R. Cheeke; N.M. Patton and W.H. Kennick (1996). Carcass and meat characteristics of Flemish Giant and New Zealand White purebred and terminal cross rabbits. J. Applied Rabbit Res. 4: 66.

Szymczyk B. and W. Szczurek (2016). Effect of dietary pomegranate seed oil and linseed oil on broiler chicken's performance and meat fatty acid profile. Journal of Animal and Feed Sciences. 25: 37-44.

Thomsen P.S.; T.M. Jensen; K.A. Hammer; C.F.Carson; P. Mølgaard; T.V. Riley and P.S. Thomsen (2011). Survey of the antimicrobial activity of commercially available Australian tea tree (Melaleuca alternifolia) essential oil products in vitro. J Altern Complement Med. 17(9): 835-841.

Verardo V., P. Garcia-Salas, E. Baldi, A.S. Carretero; A.F. Gutierrez and M.F. Caboni (2014). Pomegranate seeds as a source of nutraceutical oil naturally rich in bioactive lipids. Food Res. Int. 65:445–452.

Vogel S.F. (1975). Fatty acid composition of raw and processed meats. Food Technol. 29: 147-152.

Walter B.M. and G. Bilkei (2004). Immunostimulatory effect of dietary oregano etheric oils on lymphocytes from growth-retarded, low-weight growing-finishing pigs and productivity. Tijdschr Diergeneeskd. 129:178–181.

Wang H.X.; W.S. Zhong and B. Guo (2011). Study of malondialdehyde (MDA) content, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in chickens infected with avian infectious bronchitis virus. Afr. J. Biotechnol., 10 (45): 9213-9217.

Wang L.; R. Tabor; J. Eastoe; X. Li; R. Heenan and J. Dong (2009). Formation and stability of nanoemulsions with ionic-nonionic surfactants. Physical chemistry chemical physics: PCCP. 11: 9772-9778.

Zeng Z.; X. Xu; Q. Zhang; P. Li; P. Zhao; Q. Li; J. Liu and X. Piao (2015). Effects of essential oil supplementation of a low-energy diet on performance, intestinal morphology and microflora, immune properties and antioxidant activities in weaned pigs. Anim. Sci. J. 86: 279–285.

Zheng W. and S.Y. Wang (2001). Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem. 49: 5165–517

تأثير إضافه مخلوط الزيوت الأساسيه المستحلبه و المستحلبه نانويا علي أداء النمو و خصائص اللحم في الأرانب الجبلي المفطومه

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أجريت الدراسه بهدف تقييم تأثيرات الأشكال المختلفة لخليط الزيوت العطريه (الثوم ، الرمان ، شجرة الشاي) ، المستحلبه والمستحلبه نانويا بنفس المستوى ، على أداء النمو ، ومكونات الدم ، وتحليل اللحوم. ، والأحماض الدهنية في اللحم ، ومؤشرات المناعة. تم تقسيم 45 من الأرانب الجبلي المفطومة ، بشكل عشوائي إلى ثلاث مجموعات متطابقة. تم تغذية الأرانب في المجموعة الأولى(T1) الكنترول بخليط العلف المركز فقط ، المجموعة الثانية (T2) والمجموعة الثالثة (T3) تم تغذيتهما بخليط العلف المركز بإضافه كلا من 0.75 مل مستحلب / كجم علف ، 0.75 مل مزيج الزيوت العطرية نانويه المستحلب / كجم عليقه ، على التوالي. سجلت نتائج أداء النموأن أعلي وزن حي نهائي لمجموعتي T2و T3 (P <0.05) مقارنة بالمجموعة المقارنة (الكنترول). سجلت المجموعتين T2 و T3 (P <0.05) أعلى وزن ذبيحة مقارنا الكنترول. و فيما يتعلق بمحتوى بروتين اللحوم للمجموعات الثلاث المختبرة سجلت المجموعه T3 القيمة الأعلى (P <0.05) ، و سجلت أيضا T3 أقل محتوى دهني ف اللحم ، TVNوTBA ،أظهرت المجموعة T1 (الكنترول) أعلى نسبة من الدهون الثلاثية والكوليسترول واليوريا والكرياتينين AST و ALT ، و كانت قيم المناعه من حيث IgG و IgM أعلى للمجموعه T3 ، سجلت المجموعه T3 أعلي مضادات أكسدة (P <0.05) من حيث GPx و SOD ، بينما سجلت المجموعه الكنترول أعلى قيما معنوية لإجمالي الأحماض الدهنية المشبعة .و تم الحصول على نتائج عكسية بخصوص الاحماض الدهنية الاحادية غير المشبعة و إجمالي الأحماض الدهنية المتعددة غير المشبعة حيث سجلت المجموعه T3 قيما معنوية أعلى.