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Synergistic Effects of Using Essential Oils Blend (garlic, onion and lemon) as a Nanoemulsion on the Productive Performance of Growing Rabbits.

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Abstract

The objective of the current study was to evaluate the effects of adding Nano- emulsified essential oil blend (NEOB) (garlic, onion, lemon essential oil) as feed additive, on growth performance, blood components ,immunity indices, meat analysis and meat fatty acids of weaned Mountain rabbits. A total of 72 male weaned mountainous rabbits breed (Gabali local breed) with an average live body weight of 590 gm were allotted randomly into 4 experimental groups 18 animals each, (each group was divided in to 3 replicates, 6 animals each). The first group was served as a control and the three other groups were fed on 0.5, 0.75 and 1 ml/kg of feed Nano emulsified natural oil blend as treatments T1, T2 and T3 respectively. Final live body weight, total weight gain, average daily weight gain and feed conversion ratio for T3 were (P<0.05) superior in its values in these measurements compared with other treatment group. On the other hand, the highest average feed intake was (P < 0.05) recorded by the control compared with T3, T2 and T1 respectively. While, rabbits of T3, T2 and T1 were (P < 0.05) higher in digestibility coefficient of CP, EE and NFE compared with the control. The same trend was observed in DCP and TDN values in favor of T3, T2 and T1 compared with the control. Rabbits of T2 and T1 were (P < 0.05) significantly higher in blood serum total protein and globulin compared with the control. Regarding to blood serum triglycerides, cholesterol and LDL, the control group recorded (P < 0.05) the highest values compared with T3, T1 and T2. In addition, T3, T2 and T1 recorded (P < 0.05) the highest values of IgG and IgM compared with the control. No (P < 0.05) significant differences were found among treatment in kidney's and liver weight. Heart weight was (P < 0.05) significantly differ among the control, T3 and T1 compared with T2. The lowest value for full weight of digestive tract was (P < 0.05) recorded by T3 and T2 compared with the control T1. T3, T2 and T1 were (P < 0.05) higher in the meat protein compared with the control. Regarding to meat pH, TVN and TBA the control group was (P<0.05) higher values compared with T3, T2 and T1. The best meat antioxidant values for GPx was (P < 0.05) recorded by T3, T2 and T1 compared with control while the control recorded (P < 0.05) the highest value of meat MAD compared with T3, T2 and T1. The control group recorded (P < 0.05) the highest values of meat total saturated fatty acids compared with T3, T2 and T1 repressively. While, T3, T2 and T1 recorded (P < 0.05) the highest total monounsaturated fatty acids compared with the control as well as values of total poly unsaturated fatty acids.

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

Introduction

Since 2006, the European Union has prohibited use of antibiotics in food preparation, in order to reduce the spread of resistant bacteria through the food chain and the growing public concern over the potential health risks and environmental effects resulting from the overuse of antibiotics as growth promoters in animal production. However, the primary producers, processors, and retailers in the animal production chain are always looking for efficient, secure, and affordable substances with comparable qualities. Therefore, the quality of the derived animals products (meat, milk, and eggs), as well as growth performance parameters have been improved by using natural feed additives from plants, such as essential oils (EOs), in animal production as alternative of synthetic feed additives **(Simitzis, *et al*, 2011)**. Aromatic plants produce essential oils as secondary metabolites, which are volatile, natural, complex compounds with a characteristic odor. They are commonly produced using steam or hydro-distillation. In particular today's pharmaceutical, sanitary, cosmetic, agricultural, and food sectors, essential oils have been used widely for, viruscidal, bactericidal, anti-parasitic, fungicidal, insecticidal, cosmetic and medicinal uses., and also, essential oils (EOs) are natural extracts with folk medical origins. In general, their usage is environmentally friendly, beneficial, non-toxic, and in line with nature. Using essential oils as natural alternative to replace synthetic antibiotic are now being evaluated. Some of these essential oils have high antibacterial properties as well as other beneficial benefits on the feeding of monogastric animals **Pavel Horky *et al.* (2019)**.

Gebreyohannes, *et al.*, (2013) reported that since ancient times, the Amaryllidaceae family member garlic (*Allium sativum*) has attracted the attention as potential medical and therapeutic materials. **Liu *et al.*, (2012)** reported that garlic essential oil (*Allium sativum L.*) contains flavone, tannins, quinine, alkaloids, terpenoids, saponins, polyphenols, esters, flavonoids and on-volatiles

residues. These substances have many useful effects; anti-coccidail, antimicrobial, antioxidants and digestive enzyme enhancer, for better utilization of nutrients to improving digestion, liver function and absorption.

Onions (*Allium cepa*) are characterized by flavanol quercetin component and its derivatives that give onions their distinguishing flavor. It is also, contain abundant other bioactive substances, including fructo-oligosaccharides and Sulphur compounds **(Roldán *et al*., 2008)**. Onion seeds found to have significant percentages of oil (21.86%–28.86%), according to a physio-chemical examination. According to gas chromatography technique (GC) data, linoleic acid account for (49.4 - 60.66%) of onion seed oil (49.42–60.66%), followed by oleic and palmitic acid. **Hasan and Hatice (2014)** stated that numerous of chemicals were discovered, including alcohols, acids, hydrocarbons, esters, and compounds containing Sulphur. According to **Dima Mnayer *et al*. (2014)** the primary constituents were (30.92%) di-propyl disulfide, (17.10%) di-propyl tri-sulfide, (7.26%) 1-propenyl propyl disulfide, and (5.20%) methyl propyl tri-sulfide. Di-propyl disulfide and di-propyl tri-sulfide reported to be the two main substances found in onion essential oil**.** Their Sulphur compounds are primarily responsible for their defined antibacterial and antioxidant properties **(Kim *et al*, 2004)**.

Omani sour lemon (*Citrus limon*) essential oil contains (53.57%) limonene, (14.69%) a-terpineol, (8.23%) pinene, (1.84%) a-pinene, (1.51%) b-myrcene, (4.33%) terpinolene, (3.38%) terpinen-4-ol, (1.80%) cymene, (1.43%) b-bisabolene, (0.85%) b-linalool and (1.08%) E-citral. **Tadtong *et al*. (2015)** reported that sour essential oil contains chemical compounds such as (53.57%) limonene, (15.15%) a-terpineol, (7.44%) b-pinene, (4.33%) terpinolene, (3.55%) terpinen-4-ol, cymene (2.88%) and E-citral (2.38%).

The ultimate beneficial nanotechnology applications in the field of animal nutrition are essential oil Nano emulsions. They are emulsions with droplet diameters ranging from 20 to 100 nm. **(El-Sherbiny *et al*., (2016).** The Nano-emulsified EOs had a greater effect in increasing fatty acids (n-3 and n-6). The biohydrogenation rate of polyunsaturated fatty acids into saturated fatty acids was reduced by Nano emulsified EOs. Supplements containing Nano emulsified oil were more effective than supplements using raw oils **(El-Sherbiny *et al*., 2016; Mousa *et al*., 2022 and Sherein H. Mohamed and Walaa M. Abd El-Wahab 2022**).

The objective of the current study was to evaluate the effects of adding Nano- emulsified essential oil blend (NEOB) (garlic, onion and lemon essential oil) as feed additive, on growth performance, blood components, immunity indices, meat analysis and meat fatty acids of weaned Mountain (Gabli) rabbits

Materials and Methods

Preparation of Nano-emulsion oil mixture

The Nano-emulsion was produced by mixing 15% oil (5% garlic, 5% onion, and 5% lemon) and 5.6% surfactant (Tween 80), followed by 79.4% distilled water. The nano-emulsion mixture was formed using a 1000 rpm magnetic stirrer at 25 C° for 10- 20 minutes. The Nano-emulsion formulation was done according to the method described by **Ragavan *et al*. (2017), Kentish *et al*. (2008) and Sherein H. Mohamed and Walaa M. Abd El-Wahab 2022)**

Characterization of Nano-emulsion

Potential zeta

To obtain a homogeneous electrical charge between emulsions particles in order to avoid re-aggregation of oil particles as long as feasible, Laser Doppler electrophoresis was used to determine the surface charge of the Nano emulsion **(SZ-100, Horiba Scientific, Kyoto, Japan)**. Deionized water was used to dilute the samples before injecting them into a capillary cell to test the charge at 25 C°, with zeta potential values measured in millivolts. As a result, an apparent zeta potential picture is produced (Figure 1.).

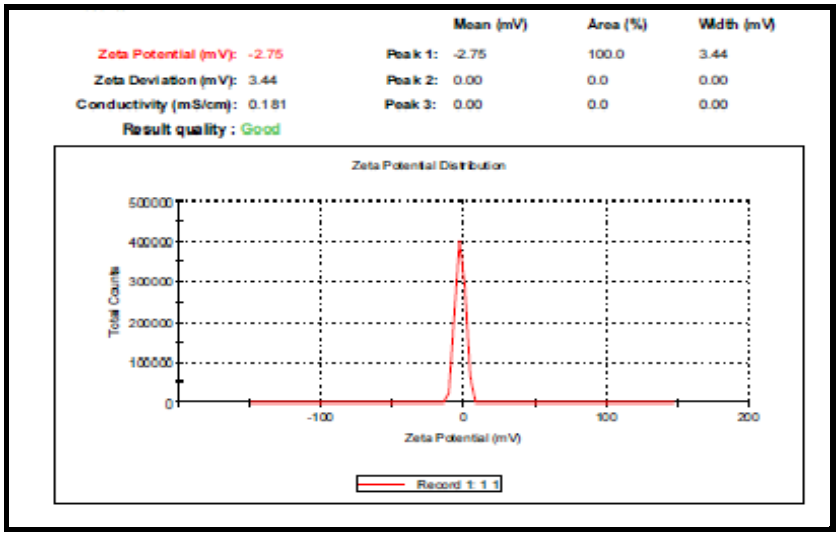

Figure 1: Nano-Emulsified Essential Oil Blend's Zeta Potential

Transmission electron microscopy (TEM)

The EOs mix Nano-emulsion structure and shape were examined using the TEM (**FEI-TECNAI G2- 20 TWIN, Netherland)**. The Nano-emulsions were placed on carbon film-coated 300 mesh copper grids after being diluted with deionized water at 10 and 100-fold small drops. The grid was TEM inspected at 80 kV after drying for three hours in vacuum resulting in a positive view (Figure 2.).

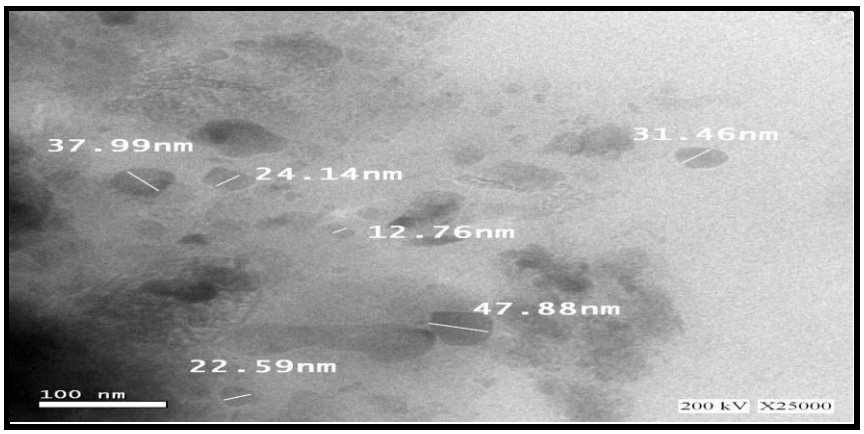

Figure2: photos taken with a transmission electron microscope of a mixture of essential oils at nanoscale (garlic, onion and lemon essential oil).

Animals and experimental design

A total of 72 weaned male mountainous rabbits breed (local Gabali breed) with an average live body weight of (393.01±1.02 g) were allotted randomly into 4 experimental groups 18 animals each (each group was divided in to three replicates, 6 animals for each). The first group was served as a control and the three other groups were fed on 0.5, 0.75 and 1 ml/kg of feed Nano emulsified natural oil blend (mix of garlic, lemon and onion) as T1, T2 and T3 treatments, respectively. A basal ration was formulated as shown in Table 1 which used as a control. Three rations were also prepared by adding 0.5, 0.75 and 1 ml Nano emulsified oil blind per each kg of control feed and served as 3 experimental treatments, The emulsified nano-oils were sprayed directly on the pelleted rabbits feed, then dried and stored in a clean, well-ventilated space till use in the experiment.

Table 1. Composition and chemical analyses of basal ration

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	
Digestible energy(kcal/kg)	1465.44
CP%	20.42
CF%	14.05
EE %	1.73
Calcium%	1.38
Ph%	0.46
Lysine%	1.16
Meth.+ Cyst. %	0.58

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

Growth performance trial

The feeding trial was conducted at animal production experimental farm of Faculty of Agriculture, Moshtohor, Benha University. An orientation period for one week was conducted before the start of feeding trial as a adaptation period to apply gradual change feeding system from milk feeding of young rabbits to dry feeding to avoid after weaning chock using barley and dry experimental feed. The growth or the feeding trial started at first of August 2021 and lasted for 12 weeks. Feed requirements of experimental rabbits were adjusted according to Ministerial decree of Agriculture Ministry Decree (1996). Every two weeks, the experimental animals' weights were taken, and feed needs were changed. Fresh water was offered free all day long. Once a week at 9:00 am, a digital thermometer was used to measure and to record animal's rectal temperature as well as respiration rate while animals were constant and relaxed.

Digestibility coefficients trial:

sixteen rabbits (4 from each treatment) were used in digestion trial to assess the apparent digestible coefficient and nutritional value of the experimental diets after feeding study was completed. During the collecting period of four days, a plastic net was placed under the cages to collect non-urine waste, and 2% boric acid was sprayed on the nets to capture the ammonia. The feces were crushed after being dried in an oven at 60 c to get two consistent weights during the collecting period. Following that, feed and feces samples were chemically examined in accordance with **A.O.A.C (2005)** to determine the nutritional value and nutrients digestion coefficients for each dietary treatment as stated by **Abou Raya *et al*., (1974)**.

Carcass trail:

Sixteen animals (four animals from each group) were slaughtered at the end of the feeding trial. Live body weight of the animal, the front and back legs, the head, the hot hairless carcass, gut, liver, lungs, kidneys, the rear parts, the shoulders and the ribs were weighed and recorded. The dressing % was calculated based on **Steven *et al*., (1996)**.

Chemical composition and Antioxidant capacities determination of rabbit's meat:

Analysis of meat samples of experimental rabbit was conducted to determine its chemical composition (moisture, fat, protein and ash), according to **AOAC (2005)**. Total volatile nitrogen (TVN) was evaluated in accordance with Egyptian Organization for Standardization (ES: 63/9/2006), while the pH of the meat was calculated in accordance to Pearson (2006) as following formula:

TVN/l00g = (ml H_2 So_4 n0.1 for sample - ml H_2 So_4 n0.1 for blank) x 14

Malonaldehyde (MDA), a byproduct of lipid peroxidation, was analyzed to determine the thiobarbituric acid number (TBA) and its associated value (s) by spectrophotometer **(UNICAM969AA Spectronic, USA)** according to Egyptian Organization for Standardization **(ES:63/10/2006)** method to test the sample's absorbance at wavelength 538 as following formula:

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

According to **Fang *et al.* (2011)**, Glutathione Peroxidase (GPx) was determined commercially using GPx kits (Randox, Crumlin, UK). Malondialdehyde (MDA) levels in rabbit serum were determined and calculated and represented as U/mg protein according to **Wang *et al*., (2011)**.

Rabbit's meat fatty acid fractionation:

According to **Aura *et al*., (1995)**, fatty acids (FAs) were determined in rabbit's meat using the gas chromatography technique (GC). Fat extraction was performed according to **AOAC (2005)**.

The extracted FAs were diluted in 0.5–1.0 ml anhydrous diethyl ether, and the yellow hue was maintained by adding drops of diazomethane solution until the methylation process was completed **(Vogel 1975)**. Hewlett Packard gas chromatography (5890 series) with a flame ionization detector was used to conduct the GC analysis.

Blood serum parameters:

Four rabbit's blood samples from each group were taken during slaughter trial representing each experimental treatment. A five ml of blood sample was collected from each rabbit into a clean and dry tube without the addition of anticoagulants. To get blood serum, samples were then centrifuged at 3500 pm. for 20 minutes, then serum was divided into 2-ml sample and placed into clean, dry Eppendorf tubes and stored at 20 °C to be used later for further examination. The serum total protein was determined using the **Armstrong and Carr (1964)** technique, and albumin was determined according to **Doumas *et al*., (1971)**. Globulin was calculated by subtracting determined albumin from total protein. Serum cholesterol was determined and expressed as mg/dL according **Rolschlau (1974).** The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes were determined according to **Reitman and Frankel (1975)** and expressed as IU/L. Creatinine was determined by **Julian (2000)** approved method using the readily accessible commercial kits offered by Biomerieux, France, and expressed as mg/dL. **Fossati and Prencipe (1982)** method was used to determine the triglycerides in rabbit serum. According to **Patton and Cronch, (1979)** the blood urea nitrogen was measured using an enzymatic colorimetric method. As well as following the instructions, the commercial bio diagnostic kits from Bio Diagnostic Company (Giza, Egypt) and a spectrophotometer **(**Shimadzu, Japan) were used to measure the immunoglobulin G (IgG) and immunoglobulin M (IgM) levels in the produced rabbit serum.

Statistical analysis

Data were statistically analyzed by ANOVA (one-way analysis) using **SAS (2013)** procedures. Duncan's multiple tests **(Duncan, 1955)** were used for mean comparisons.

Results and discussion

Effect of NEOB on digestibility, nutritive values of experimental treatments.

As given in Table (2) no significant ($P > 0.05$) differences were detected among all test groups in digestible DM and digestible OM and digestible CF while T3, T2 and T1 were ($P<0.05$) higher in digestibility coefficient CP, EE and NFE compared with the control. The same trend was observed in DCP and TDN values for rabbits of T3, T2 and T1 compared with the control. **Shehata *et al*. (2003)** and **Hernandez *et al*., (2004)** indicated that the great improvement in CP and CF digestibility from adding garlic with different levels. **Muhl and Liebert, (2007)** reported that dietary essential oils improve the performance of birds, because these substances stimulate the secretion of internal digestive enzymes consequently, increases the digestibility of nutrients, the rate of gut passage and feed intake. Furthermore, for modulating the gastrointestinal eco-system or having antimicrobial activities, the essential oil affects the digestibility of protein, starch and fat in gastrointestinal tract **(Hernández *et al*. 2004; Lee *et al*., 2004)**. **Sayed Ahmed, *et al*., (2019)** reported that dietary supplementation with moringa , onion oils or mixture of both found to have ($P < 0.05$) significantly effects on digestibility coefficient of CP and EE, being the highest was for the mixture of both onion and moringa oil, followed by onion oil ration.

Table 2. Digestibility, nutritive values of experimental treatments**.**

Item	**Treatments**				
	Control	**T1**	**T2**	**T3**	**±SE**
Digestibility coefficients %					
DM	66.31	67.94	68.72	69.09	0.96
OM	65.69	67.39	68.14	68.66	1.00
CP	79.03^{b}	81.02^{ab}	81.82^{a}	82.70^{a}	0.77
CF	67.13	68.74	69.49	69.86	0.94
EE	83.43^{b}	87.90^{a}	88.19^{a}	89.75^{a}	0.58
NFE	73.51^{b}	73.21^{b}	76.04^{a}	76.67^{a}	1.39
Nutritive value %					
digestible crude protein (DCP)	15.81^{b}	16.21^{ab}	16.36^{a}	16.54^{a}	0.15
Total digestible nutrient (TDN)	81.28^{b}	83.52^{ab}	84.29^{a}	85.08^{a}	0.79

a,b,c, mean within some rows with differing superscript are significantly differ (P<0.05).

Effect of NEOB on growth performance of tested animals at total experimental period

Results of table (3) showed significant (P<0.05) differences between the control and T3 in the initial live body weight. In the meantime, no significant (P>0.05) differences were observed between the control and T1 and T2 and T3 in the initial live body weight. Regarding to final live body weight, total weight gain, average daily weight gain and FCR T3 were (P<0.05) superior compared with T2, T1 and the control. In the meantime, T2 and T1 were ((P<0.05) better in those traits compared with the control. On the other hand the highest average feed intake was (P<0.05) recorded by the control compared with T3, T2 and T1, respectively and the lowest feed intake (P<0.05) was recorded by T3 compared with T2, T1 and the control respectively.

Table 3. Effect of NEOB on growth performance of tested animals at total experimental period

Items	**Treatments**				
	Control	T1	T2	T3	±SE
Initial live body weight (g)	595	580	590	590	1.20
final live body weight(g)	1720^{d}	1910^{c}	1980^{b}	2040^{a}	8.12
Total weight gain(g)	1125^{d}	1330^{c}	1390^{b}	1450^{a}	8.25
Average daily weight gain(g)	12.5^{d}	14.77^{c}	15.44^{b}	16.11^{a}	0.55
Average feed intake(g)	88.32^{a}	81.61^{b}	81.34^{c}	80.91^{d}	0.71
Feed conversion ratio	7.06^{a}	5.52^{b}	5.26^{c}	5.02 ^{d}	0.03

a,b,c, mean within some rows with differing superscript are significantly differ (P<0.05).

In agreement with these results, essential oils (EOs) improved the growth performance parameters and the quality characteristics of the derived products (meat and milk), **(Simitzis, *et al*., 2011)**. **Liu *et al*., (2012)** reported that garlic essential oil (*Allium sativum L*.) contains of flavone, tannins, quinine, alkaloids, terpenoids, saponins, polyphenols, esters, flavonoids and on-volatiles residues. These substances have many useful effects as anti-coccidail, antimicrobial, antioxidants and digestive enzyme enhancer, to improve the utilization of nutrients to improving digestion, liver function and absorption. **El-Wafa, *et al.* (2002)** reported that feeding dried garlic for 8 weeks induced significant improvement in daily live weight gain and body weight of rabbit. Using phenolic-rich onion extract as a feed additive in broiler chicken diets can improve their growth performance in term of increase in average daily feed intake, average daily gain and body weight in a dose-dependent manner by improving AID% of amino acids as well as integrating the intestinal histology.

Jamroz, *et al*., (2005) reported that inclusion of essential oils (OEs) in birds feed improve their growth performance for birds which stimulates secretion of digestive enzymes, subsequently, improved the rate of digesta passage and nutrient digestion. Presence of bioactive compounds; anetole, eugenol, thymol and carvacrol in respective EO are responsible for improving appetite sense which ultimately increases feed intake **(Ertas, *et al* 2005). Nomeary, *et al*., (2020)** found that garlic EO significantly (P<0.05) increased the total body weight and the average body weight gain of New-Zealand white rabbits by 12.4% for both, compared to the control group. This improvement in live body wight with phytogenic additives supplementation may also be attributed to: a. the provision of some compounds that increase the digestion and absorption of certain nutrients in the diets, b. the bioactive ingredients (curcuminoids and allicin) present in garlic that cause greater efficiency and also in enhanced growth. In partial agreement to results of the current study **Gbenga, *et al*., (2009)** indicated that feed conversion, body weight gain and feed intake were not statistically influenced by dietary garlic supplementation; they also observed that the animals consumed high concentration of garlic supplement recorded slight increase in body weight gain compared to the control fed animals.

Diya AL-Ramamneh (2017) reported that chicken fed diet supplemented with onions at level of 5%, achieved significant improvement in chicken performance of body weight. **Aji, *et al*. (2011**) stated that it is possible to mention dietary onion could improve the growth performance of chicks, due to its content of organo-sulphur compounds that reduce the growth of some pathogenic bacteria in the gastrointestinal tract of broilers. **Abou El-Wafa *et al*., (2002)** reported that daily weight gain and live body weight and feed conversion ratio were significantly improved by adding onion oil to rabbit's diet.

Khattak *et al*., (2014) reported that 7% improvement in live weight gain when supplementing lemon essential oil at the dose of 100 mg/kg feed. Supplementation of lemon peel essential oil in broiler diets has resulted in the increase in live weight gain by 8.77% over control birds **(Sahu, *et al*., 2019)**. **Tiihonen, *et al*., (2010)** reported that supplementation broiler diets with 5 g/t cinnamaldehyde and 15 g/t thymol (essential oils Eos) in chicks' diet resulted in decrease in undesirable bacterial growth, thus maintaining a healthy gut microbiota and improving growth performance. Using 5% onion extract showed better FCR compared with control group (1.04±0.13, 1.08±0.11 respectively)

Effect of NEOB on blood serum characteristics of experimental treatments

As shown in table (4) statistical analyses for the effect of NEOB on blood serum constituents of experimental animals showed that T3, T2 and T1 were (P<0.05) significantly higher in blood serum total protein compared with the control. Similar trend was observed regarding to values of blood globulin. While differences in the values of albumen among T3, T2 and the control were not (P<0.05) significant. Regarding to blood serum triglycerides, blood cholesterol and LDL, the control group recorded (P<0.05) higher values compared with T3, T1 and T2. On the other hand T3, T2 and T1 recorded (P<0.05) higher values of blood HDL compared with the control. The control group recorded (P<0.05) values of blood serum urea, creatinine, AST and ALT compared with T3, T1 and T2. While T3, T2 and T1 recorded higher (P<0.05) values of IgG and IgM compared with the control. Similar results were obtained by **Hassan and Abdel-Raheem (2013)** who reported that garlic can increase theblood total protein, albumin and globulin concentrations. Also, **Goodarzi, *et al*., (2013)** found that broiler chicks fed 30 g/kg onion juice achieved higher body weight gain and serum high density lipoprotein (HDL)-cholesterol while triglyceride level were decreased. Other studies reported significant lower in serum total cholesterol by dietary quercetin and onion extract because onion contains sulfur organic compounds including S-Methylcysteine sulfoxide and S-allyl cysteine sulfoxide and these compounds are related to decreasing of liver protein, glucose and blood lipid, as reported by **(Qureshi *et al*., 2011; Melvin and Jayachitra, 2009 ; An BK *et al*., 2015)**. Also **Hussein *et al*., (2007)** found that garlic and onion oils improved serum total protein and albumin. While **Bordia *et al*., (1977)** suggested that the essential oils of garlic and onion have protect potential against experimental atherosclerosis by preventing the fall in the alpha lipoprotein fraction and by enhancing fibrinolytic activity, as well as by

lowering the serum cholesterol and triglyceride levels. The increase of globulin (Glb) concentration may be an indicator to increase immunity in rabbits since the liver will be able to synthesize enough globulin for immunologic action as mentioned by **Sunmonu and Oloyede (2007)**. **Yu *et al.* (1994)** reported that essential oils (Eos) supplementation was found to decrease cholesterol levels since the bioactive compounds present in EOs, such as geraniol, enchone, menthol, fenchyl alcohol, cineole, borneol, citral, menthone and ionone, are essential in inhibiting hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) activity a crucial enzyme in the synthesis of cholesterol.

Table 4. Blood characteristics of experimental treatments

Item	**Treatments**				
	Control	**T1**	**T2**	**T3**	**±SE**
Blood constituents					
Total protein (mg/dl)	4.65^{c}	5.50^{b}	6.20^{a}	6.775^{a}	0.21
Albumin (mg/dl)	3.00^{a}	2.475^{b}	2.70^{ab}	2.975^{a}	0.13
Globulin (mg/dl)	1.65^{b}	3.025^{a}	3.55^{a}	3.80^{a}	0.33
Tri glycerides (mg/dl)	74.80^{a}	64.825^{b}	61.125^{bc}	57.70^{c}	1.40
Cholesterol (mg/dl)	59.50^{a}	53.50^{b}	50.725^{b}	46.60^{c}	1.31
HDL (mg/dl)	31.425^{c}	33.725^{b}	34.625^{ab}	35.525^{a}	0.51
LDL (mg/dl)	28.075^{a}	19.775^{b}	16.10^{bc}	11.0250^{c}	1.78
Urea (mg/dl)	28.825^{a}	23.525^{b}	21.575^{b}	18.55^{c}	0.68
Creatinine (mg/dl)	0.8175^{a}	0.705^{b}	0.6275^{c}	0.5725^{c}	0.02
AST (U/L)	38.30^{a}	32.075^{b}	29.25^{c}	25.650^{d}	0.88
ALT (U/L)	21.30^{a}	19.425^{ab}	17.65^{bc}	15.80^{c}	0.62
IgG (mg/dl)	32.65^{d}	35.50^{c}	38.30^{b}	41.275^{a}	0.64
IgM (mg/dl)	50.05^{c}	59.325^{b}	62.20^{b}	67.25^{a}	1.01

a,b,c, mean within some rows with differing superscript are significantly differ (P<0.05).

4.1. Effect of NEOB on carcass characteristics of experimental treatments

As given in Table (5) no (P > 0.05) significant differences between the control and T1, T2 and T3 in kidney's and liver weight were detected. While heart weight was (P<0.05) significantly differ among the control, T3 and T1 compared with T2. The lowest value (P<0.05) for full weight of digestive tract was recorded by T3 and T2 compared with the control and T1. Regarding to rack weight, loin weight of T3 and the control were (P<0.05) higher than other treatments. In line with the obtained results **Sayed Ahmed, *et al.*, (2019**) reported that hot carcass weight and dressing percentage were significantly (P≤0.05) higher for rabbits fed diets supplemented with onion oil as compared with those fed the control diet.

Table 5. Carcass **characteristics of experimental treatments**

Item	**Treatments**				
	Control	**T1**	**T2**	**T3**	**±SE**
Carcass in (g)					
Kidneys weight	18.75	18.75	17.15	16.53	1.06
Liver weight	68.25	73.75	72.75	68.25	3.26
Heart weight	11.25^{a}	9.75^{ab}	7.60^{b}	8.95^{ab}	1.03
Digestive tract full weight	390.00^{ab}	426.25^{a}	371.25^{b}	370.00^{b}	12.19
Hind up	133.75^{a}	116.25^{b}	130.00^{a}	123.75^{ab}	4.10
Shoulder weight	208.75^{ab}	211.25^{a}	200.00b	192.50^{c}	9.26
Rack weight	200.00^{a}	158.75^{b}	173.75^{ab}	197.50^{a}	11.19
Loin weight	221.25^{a}	210.00^{b}	203.75^{c}	218.75^{a}	14.65
Round weight	415.00^{a}	391.25^{bc}	380.00^{c}	402.50^{b}	16.80
Hot Carcass weight	1157.50^{a}	1055.00^{b}	960.00^{c}	1048.75^{b}	7.70
Lung and Trachea weight	17.50^{a}	12.50^{b}	15.85^{ab}	12.46^{b}	1.43
Leg weight	71.25	75.00	71.25	72.50	3.72
Belt weight	315.00^{a}	286.25^{b}	287.50^{b}	275.00^{c}	16.39
final weight	2012.50^{d}	2108.75^{b}	2050.00^{c}	2200.00^{a}	11.78

a,b,c, mean within some rows with differing superscript are significantly differ (P<0.05).

Effect of NEOB on chemical analyses of meat and antioxidant of experimental treatments.

Values of meat chemical analyses of the experimental animals are shown in Table (6) Statistical analyses revealed that the highest ($P < 0.05$) moisture and fat content were recorded by the control group compared with other treatment, while rabbits of T3 was significantly higher in the meat protein compared with the other treatment group. The lowest ash content was ($P<0.05$) recorded by the control compared with T3, T2 and T1. Regarding to meat pH and TVN and TBA the control group was significantly ($P<0.05$) higher in these values compared with T3 followed by T2 and T1. Regarding to the meat antioxidant, the best value for GPx was ($P<0.05$) recorded by T3, T2 and T1. while the control group recorded ($P<0.05$) the highest value of meat MAD compared with T3, T2 and T1. These results could be attributed to the fact that, the major compounds presented in onion EO are di-propyl disulfide and di-propyl tri-sulfide as stated by **Kim, *et al*, (2004a)** that have been reported to have anti-oxidant and anti-microbial activities which are mostly associated with their sulfur compounds. Several previous studies by **(Arjmand and Dastan, 2020)** have highlighted that EOs, and their active components are important as an anti-inflammatory, anti-oxidant, antimicrobial, anti-parasitic and as immune modulators. Furthermore, natural substances that have antioxidant activity can be defined as molecules able to react with free radicals **(Elshafie and Camele., 2017)**. Regarding the mechanism of action of EOs as antioxidant, several studies have highlighted that terpenoids belong to phenolic group such as thymol; eugenol, carvacrol, and methyl chavicol are the main components responsible for EOs antioxidant activity where they have to donate hydrogen or an electron to free radicals, shielding other biological molecules from oxidative stress as mentioned earlier by **Jugreet *et al*., (2020)** that could support the obtained results shown in Table 5. **Rahimi *et al*., (2011)** reported that EOs are rich source of such phenolic, natural antioxidant compounds. EOs are also, have positive impact on antioxidant expression, such as glutathione peroxidase, catalase and superoxide dismutase (SOD), and prevent off-flavor polyunsaturated fatty acid oxidation and the formation of reactive oxygen species (ROS) **(Marcincak *et al*., 2008; Miguel, 2010)**. Furthermore, **Habibi *et al*., (2014)** showed that ginger EOs supplementation at 150 mg/kg increased the total SOD activity and decreased the malondialdehyde (MDA) levels in the liver in broiler chicks.

Table 6. Chemical analyses of meat and anti-oxidant of experimental treatments

Item	Treatments				
	Control	T1	T2	T3	±SE
Meat analyses					
Moisture%	73.675^{a}	72.875^{b}	73.125^{b}	72.775^{b}	0.18
Protein%	19.575^{c}	20.275^{bc}	20.750^{ab}	21.45^{a}	0.29
Fat%	2.85^{a}	2.35^{b}	2.175bc	1.875^{c}	0.11
Ash%	1.825^{c}	2.20^{b}	2.325^{ab}	2.6250^{a}	0.11
pH	5.765^{a}	5.7075^{a}	5.6375^{b}	5.59^{b}	0.02
TVN (mg/Kg)	5.35^{a}	4.00^{b}	3.450^{b}	2.025^{c}	0.29
TBA (mg/Kg)	0.190^{a}	0.125^{b}	0.100^{cb}	$0.0\ 575^{c}$	0.02
GPx(U/mg protein)	415.25^{c}	462.50^{b}	480.50b	513.25^{a}	8.80
MDA (nmol/mL)	1.80^{a}	1.50^{b}	1.325^{bc}	1.200^{c}	0.08

a,b,c, mean within some rows with differing superscript are significantly differ (P<0.05).

Effect of NEOB on meat fatty acids fractionation of experimental treatment's animal

As shown in Table 7, the control group recorded ($P < 0.05$) the highest values of meat total saturated fatty acids (Lauric, myristic, palmitic and stearic acids) compared with T3, T2 and T1 repressively, while T3, T2 and T1 recorded ($P < 0.05$) higher total mono unsaturated fatty acids compared with the control as well as values of total poly unsaturated fatty acids. The highly significant values of total mono-unsaturated Fatty acids and total poly-unsaturated fatty acids recorded by groups fed NEOB compared with the control could be attributed to the benefits of feeding rabbits on NEOB. In a study by **Goodarzi *et al*. (2013)** on broiler chicks fed 30 g/kg onion juice found that body weight gain and serum high density lipoprotein (HDL)-cholesterol were significantly increased while, triglyceride levels were decreased. For meat fatty acids, garlic oil is found to be an active against fat infiltration of liver **(Sang *et al*., 1995)**.

Table 7. Meat fatty acids Fractionation of experimental treatment's animal

Item	Treatments				
	Control	**T1**	**T2**	**T3**	**±SE**
Fatty acid - (mg/100g) meat					
Lauric acid (C12:0)	37^{a}	28^{b}	24^{c}	17^{d}	0.37
Myristic (C14:0)	79^{a}	65^{b}	63^{b}	57^{c}	0.84
Palmitic (C16:0)	1281^{a}	1146^{b}	1129^{c}	1094^{d}	0.80
Stearic (C18:0)	569^{a}	531^{b}	508^{c}	475^{d}	0.23
Total Saturated F.As	1966^{a}	1770^{b}	1724^{c}	1643^{d}	1.30
Palmitoleic (C16:1)	532^{d}	369^{c}	372^{b}	401^{a}	0.35
Oleic (C18:1)	1486^{d}	1543^{c}	1563^{b}	1607^{a}	6.10
Total Mono-Unsaturated F.As	1838^{d}	1912^{c}	1935^{b}	2008^{a}	6.02
Linoleic (C18:2)	612^{d}	646^{c}	659^{b}	687^{a}	0.68
Linolenic (C18:3)	135^{d}	151^{c}	172^{b}	179^{a}	0.27
Eicosadienoic acid (C20:2)	15^{d}	17^{c}	18^{b}	20^{a}	0.27
Dihomo-γ-linolenic (C20:3)	21^{d}	24^{c}	26^{b}	31^{a}	0.33
Arachidonic (C20:4)	144^{d}	160^{c}	168^{b}	183^{a}	0.44
Eicosapentaenoic "EPA" (C20:5)	10^{b}	10^{b}	14^{a}	14^{a}	0.86
Docosapentaenoic "DPA" (C22:5)	14^{c}	16^{bc}	$19a^{b}$	22^{a}	1.40
Docosahexaenoic "DHA" (C22:6)	14^{c}	16^{bc}	19^{ab}	22^{a}	1.40
Total Poly-Unsaturated F.As	970^{d}	1043^{c}	1097^{b}	1161^{a}	3.55

a,b,c, mean within some rows with differing superscript are significantly differ (P<0.05).

Conclusion

Nano emulsified essential oil blend could 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 support the recommendation of supplement rabbit's diet with **NEOB** (garlic, onion, lemon essential oil) blend at 1 ml **NEOB** /kg diet. Also, raising awareness of the application of nanotechnology in animal nutrition at the level of animal production farms.

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التأثيرات التآزرية لإستخدام مزيج الزيوت العطرية (الثوم والبصل والليمون) كمستحلب نانوي على الأداء الإنتاجي للأرانب النامية.

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تهدف الدراسة الحالية الى تقييم تاثير خليط من الزيوت الطبيعية الضرورية المستحلبة (الثوم , البصل والليمون) المعاملة بتقنية النانو كإضافة علفية على اداء النمو, مكونات الدم, الحالة المناعية و التركيب الكيماوى ومحتوى اللحم من الاحماض الدهنية فى الارانب الجبلى المفطومة. تم استخدام عدد 72 ارنب حديث الفطام بمتوسط وزن 590 ±1.02 جرام وتم توزيعها توزيعا عشوائيا على عدد اربع معاملات 18 ارنب لكل معاملة وتم تقسيمها الى 3 مكررات 6 ارنب بكل مكرر. تم استخدام مجموعه ضابطة للمقارنة واما المجموعات الاولى والثانية والثالثة فقد تم استخدامها كا معاملات تم تغذيتها على نسب 0.5 و 0.75 و1 مليجرام مخلوط (1:1:1) مستحلب من الثلاث زيوت (الثوم, البصل والليمون) المعاملة بتقنية النانو لكل كيلو جرام من العليقة الاساسية على الترتيب. اظهرت نتائج الدراسة انه توجد فروقا معنوية عند مستوى ($P<0.05$) بين مجموعة المقارنة والمعاملة الاولى والثانية وبين المعاملات الثلاث الاولى والثانية والثالثة فى متوسط وزن الارانب عند بداية التجربة. حققت المعاملة الثالثة معنويا افضل قيم للوزن النهائى لجسم الارانب, الزيادة الاجمالية فى الوزن , متوسط الزيادة اليومية فى الوزن ومعامل التحويل الغذائى مقارنة بالمعاملة الثانية والثالثة والمجموعة الضابطة. وفى نفس الوقت حققت المجموعة الضابطة معنويا عند مستوى ($P<0.05$) اعلى معدل لاستهلاك العليقة مقارنة بالمجموعة الثالثة ثم الثانية ثم الاولى على الترتيب. حققت مجموعات المعاملات الثالثة والثانية والاولى معنويا افضل معاملات هضم للبروتين والدهن والمستخلص الخالى من النيتروجين مقارنة بالمجموعة الضابطة. وتم ملاحظة وجود نفس الاتجاه لتفوق مجموعات المعاملات الثلاث معنويا بالنسبة لقيم البروتين المهضوم والمركبات الكلية المهضومة مقارنة بالمجموعة الضابطة. حققت المعاملة الثانية والاولى معنويا اعلى قيم للبروتين الكلى والجلوبيولين بالدم مقارنة بالمجموعة الضابطة. بالنسبة لقيم الدهون الثلاثية والكلوستيرول والكلوستيرول الخفيف فى الدم كانت معنويا اعلى بالمجموعة الضابطة مقارنة بالمعاملات الثلاث. حققت مجموعات المعاملات الثالثة ثم الثانية ثم الاولى معنويا افضل قيم لمكونات المناعة بالمقارنة بالمجموعة الضابطة. لم توجد فروقا معنوية فى اوزان الكبد والكلى بين المجموعة الضابطة والمعاملات الثلاث بينما كانت الفروق معنوية فى وزن القلب بين المجموعة الضابطة والمعاملات الثلاث.كان اقل وزن للجهاز الهضمى الكلى معنويا قد تحقق بالنسبة للمجموعة الثالثة ثم الثانية مقارنة بالمجموعة الضابطة والمجموعة الاولى من المعاملات. حققت المجموعة الضابطة معنويا اعلى قيم لدرجات pH TBA , TVN , باللحم مقارنة بالمعاملات الثالثة ثم الثانية ثم الاولى على الترتيب. حققت المعاملات الثالثة فالثانية فالاولى معنويا افضل قيم لمضادات الاكسدة بالمقارنة بالمجموعة الضابطة بينما حققت المجموعة الضابطة معنويا اعلى قيم MAD باللحم مقارنة بالمعاملات الثلاث. حققت المجموعة الضابطة معنويا اعلى قيم للاحماض الدهنية المشبعة الكلية باللحم مقارنة بالمعاملات الثلاث بينما حققت معنويا المعاملات الثلاث معنويا اعلى قيم للاحماض الدهنية الاحادية الكلية والمتعددة غير المشبعة باللحم مقارنة بالمجموعة الضابطة.