INFLUENCE OF NANO EMULSIFIED OREGANO, GARLIC AND CLOVE OILS BLEND ON *IN VITRO* RUMEN FERMENTATION PARAMETERS AND PRODUCTIVE PERFORMANCE OF LACTATING SHAMI GOATS.

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SUMMARY

Two experiments were carried out to evaluate the effects of supplementing different levels of nano emulsified essential oils blend (NEOB) (oregano, garlic, and clove oil) on *in-vitro* rumen gas production, rumen fermentation and lactating goats performance. The first experiment designed to study the effect on *in-vitro* gas production and rumen fermentation parameters, while, the second experiment to study the effect on lactating goat's performance trail. Fifteen Shami lactating goats, were divided into three similar groups (5 animals each). The experimental groups were (T1) the control group, were fed on (50% concentrate feed mixture :50 % roughage). While the second group (T2) and third group (T3) were fed on the control ration supplemented with 5 ml NEOB /head/day or 7.5 ml NEOB /head/day respectively. The results showed that, experimental animals which fed on NEOB groups showed reduction of *in vitro* Gas production GP (ml/200mg DM), GPSF (ml/g DM), GPSNF (ml/g DM) and SCFA (mml/ml gas) ($p<0.05$) when compared with the control group. Animals of (T3) group showed significantly ($P<0.05$) the highest values of milk yield, milk protein content, fat, TS, Ash, lactose, actual daily and fat-corrected (4% fat) daily milk yield, followed by group (T2). However, the control group (T1) recorded the lowest milk yield among all groups. Results of feed conversion ratio (FCR) in the terms of DM kg / kg 4% fat corrected milk (FCM), showed that the group supplemented with 7.5 ml NEOB/animal/day gave significantly ($P<0.05$) better values than the control group. Milk yield from animals of Group T1 recoded significantly ($P<0.05$) higher total saturated fatty acids content (SFAs), while, those of T2 and T3 recorded higher significant ($P<0.05$) values of total poly-unsaturated fatty acids ((C22:6), (C18:3), (C20:2), (C20:3), (C20:4), (C20:5), (C22:5), (C18:2)). The animals of T3 recorded ($P<0.05$) the highest albumin and globulin, immunity values in term of (IgG and IgM) and GPx (U/mg protein) antioxidant followed in a decreasing an order by T2 and T1, respectively.

Keywords: *Dairy goat; Essential oil; Gas production; Nano emulsion; un-saturated fatty acid.*

INTRODUCTION

The animal production sector around the world is under intense political and social pressure to produce safe food products with minimal use of antibiotics or synthetic origin substances (Giannenas *et al.*, 2013). Essential oils (EOs) extracted from medicinal and aromatic plants have been used as feed additives in recent years to improve the animal's productivity and general performance. These natural materials may have a variety effects, such as improved feed consumption, enhanced flavor, increased gastric, intestinal mobility, stimulation of digestive enzymes secretion (Giannenas *et al.*, 2013), antimicrobial, antifungal, antiviral, antiparasitic, immuno-modulating, antioxidant, and anti-inflammatory (Arjmand and Dastan (2020)).

Furthermore, studies recorded that EOs and their active components can inhibit ruminal methanogenesis (Benchaar *et al.,* 2008), leading to reduced methane production without affecting feed digestion. (Benchaar *et al.,* 2008 and McIntosh *et al.,* 2003). Oregano, clove, and garlic oils are important EOs that have a numerous application in a variety of practical fields specially their applications in animal nutrition.

Oregano (*Origanum vulgare*), Oregano essential oil consisted of Thymol (15.9%), Z-sabinene hydrate (13.4%), -terpinene (10.6%), p-cymene (8.6%), linalyl acetate (7.2%), sabinene (6.5%), carvacrol methyl ether (5.6%), carvacrol (3.1%) (Simirgiotis *et al.,* 2020). About 80% of oregano essential oil are obtained from thymol and carvacrol, they are mostly responsible for antioxidant efficiency (Zhai, *et al.,* 2018) Oregano essential oil and its active compounds are strong inhibitor of *in vitro* rumen methane production (Evans and Martin (2000)). Also, Oregano essential oil considered a rich source of anti-inflammatory compounds (Shen *et al.,* 2010). clove essential oil (*Eugenia caryophyllus*) consisted of 23 identified constituents, the main components in it are 76.8% eugenol, 17.4% β-caryophyllene, 2.1% α-humulene, and 1.2% eugenyl acetate (Jirovetz *et al.,* 2006). Clove and oregano essential oils are the most important EOs having a strong antibacterial activity against *Salmonella typhi* bactria, *Staphylococcus aureus* bactria, and *Pseudomonas aeruginosa* bactria (Botsoglou *et al.,* 2003). Garlic essential oil *(Allium sativum L.*) formed highly by the main components of (84.3–98.9%) sulfur compounds, (37.3–45.9%) diallyl trisulfide, (17.5–35.6%) diallyl disulfide, and (7.7–10.4%) methylallyl trisulfide, being the essential ingredients in it (Dziri *et al.,* 2014). Use of garlic and clove oils can reduce rumen methane production, without any negative effects on feed digestibility (Kamra *et al.,* 2005). Furthermore, clove and garlic EOs have antimicrobial activity against fungal pathogens (Arora and Kaur (1999). In addition, Garlic oil can reduce acetate proportion and increase propionate proportions *in vitro*, which was consistent with lower methane production (Busquet *et al.,* 2005a). Garlic oil in sheep's rations also reported to reduce methane production by 74 % by directly inhibiting rumen methanogenic bacteria, without affecting on digestibility (Busquet *et al.,* 2005b).

Nano emulsion is one of the most important nanotechnology applications. Oil nano emulsified droplet sizes ranged from 20 nm to 100 nm, Nano emulsified form had a better effect on increasing n-3 and n-6 fatty acids. Nano-emulsified oil had a positive effect on reducing the transformation rate of polyunsaturated fatty acids to saturated fatty acids in the biohydrogenation medium, all previous studies confirmed that nano emulsified oil was more effective compared to raw oils (El-Sherbiny *et al.,* 2016). Nano emulsion has a positive effect on modifying ruminant fermentation and improving the proportion of unsaturated fatty acid (UFA) in milk and meat (McCrorie *et al.,* 2011). UFA's offer potential health benefits, primarily attributed to increased CLA isomers and n-3 and n-6 fatty acids levels. Increasing the supply of UFA's in human diet's helps to prevent, or postpone the onset of atherosclerosis, coronary heart disease, inflammatory conditions, and slow the growth of tumor cells (Koba and Yanagita (2014)). The nano emulsified form also has a positive impact on reducing the rate of transformation of polyunsaturated fatty acids to saturated fatty acids in the biohydrogenation system in the rumen, this leads to reduce the methane gas emission from dairy animals and increase the net energy for milk production (El-Sherbiny *et al.,* 2016).

MATERIALS AND METHODS

This study was carried out at the Experimental goat's Farm Station at the animal research farm, Animal Production Department, Faculty of Agriculture, Benha University, Egypt. *In-vitro* gas and fermentation measurements were conducted at the feed evaluation laboratory, regional center for food and feed, Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt. Milk and Blood samples were analyzed at food analysis laboratory, Faculty of Veterinary Medicine, Benha University, Egypt. Preparation and characterization of oils nano emulsion were conducted at Nanotechnology Research Center, Egyptian Petroleum Research Institute, Nasr City, Cairo, Egypt.

1.Experimental Design and Feeding

Fifteen (15) Shami lactating goats, divided into three similar groups, 5- animals in each, and were housed in a separate pen. Each group was paired for body weight and randomly assigned to fed one of the following experimental treatments over a period of 75- experimental days. After one week of postpartum they were assigned to: T1: the control group (animals were fed ration without any supplements), T2: animals were fed the control ration plus 5 ml Nano-emulsified clove, garlic, and oregano oils blend per animal, and T3: animals were fed the control ration plus 7.5 ml Nano-emulsified clove, garlic, and oregano oils blend per animal. Each group's rations were weighed twice a day, at 7 AM and 7 PM. Feeds were determined to cover the nutrient requirements for each does according to NRC (2007). The concentrate to roughage ratio in the

ration was 50: 50%. Concentrate feed mixture ingredients presented in Table (1), while, the roughage used was Egyptian clover hay. The proximate chemical analysis of goat's feed was conducted according to the standard method recommended by Association of Official Analytical Chemists AOAC (1995). Clean drinking water was available all time.

Table (1). Feed ingredients of the concentrate feed mixture formula (CFM)

Ingredient	%
Yellow corn	40
Soybean meal 44%	10
Un-decorticated cottonseed meal	8
Wheat bran	27
Glutofeid 20%	6.5
Calcium carbonate	2
Sodium chloride (NaCl)	1.5
Molasses	5
Total (%)	100

Table (2). Chemical composition of concentrate feed mixture and Egyptian clover hay

Nutrient	CFM % on DM basis	Egyptian clover hay %
Dry matter	90.2	88.36
Organic matter	91.8	94.72
Ether extract	4.1	1.14
Crude protein	13.8	12.81
Nitrogen free extract	47	55.96
Crude fiber	26.9	24.81
Ash	8.2	5.28

2. *In vitro* Gas production and rumen fermentation parameter

Rumen fluid was collected in calibrated glass syringes before the morning feeding from three other fistulated Shami goats, that were fed twice daily at the maintenance level with Egyptian clover hay and the same concentrate feed mixture. The rumen fluid samples were transported immediately to the laboratory, then filtered well through cheesecloth. The samples were incubated using the *in vitro* technique with rumen fluid according the procedures of (Menke *et al.*, 1979). Reading of gas production was recorded at 2, 4, 6, 8, 10, 12, 14, 18, 24, 30, 36, 48, 60, 72 and 96 hrs. after incubation. Total gas levels were adjusted for blank incubation. Cumulative gas production data were fitted to the model of Orskov (1998). Digestible Organic matter DOM , digestible dry matter DDM, net energy lactation NEL (MJ/Kg DM), metabolizable Energy ME (MJ/kg DM), ME (MCal/kg DM), microbial protein MP (g/kg DOM), short chain fatty acids SCFA (mmol /ml gas), gas production structure fraction GPSF (ml/g DM), gas production non-structure fraction GPNSF (ml/g DM), digestibility crude protein intake DCPI (g/day), digestibility organic matter intake DOMI (g/day), Growth energy digestibility GED (g/Kg OMD), GED (g/Kg DMD), and TDN (%) values were calculated using equations as shown below according to Menke et al. (1979). Gas production after 24h incubation (ml/200mg-1 DM) was calculated as explained by Orskov (1998).

Equations

In vitro digestibility crude protein intake (DCPI) (G/day) = (-203.242+ (14.797*GP24+ 6.249* GP48).

In vitro digestibility organic matter intake (DOMI) (g/ day) = (-1763.07+42.5*PG24) + 13.52 * GP48).

Short chain fatty acid (SCFA) (mmol /ml gas) = (0.0239 *GP+0.0601).

Gas production structure fraction (GPSF) (ml/g DM) = (GP3h-5.5) *0.99 -3.

Gas production non- structure fraction GPNSF (ml/g DM) = (1.02*(GP24h -5.5) -(GP3h-5.5) +2).

Net energy (NE) (M cal./Lb.) = (2.2+(0.0272*gas) +(0.057*cp) +(0.149*EE)/14.64. Menke and steingass (1988).

Metabolic energy (ME) (MJ/kg DM) = 2.04+0.1448*GP+0.0036 CP+0.0243EE.

Net energy lactation (NEL) (MJ/Kg DM) = 0.08+0.1101GP+0.0022CP+0.0161.

MP = Microbial protein g/kg (DOM)= 120*DOM/100.

Organic matter digestibility OMD% = 14.88+ 0.889 GP + 0.45 CP + 0.065Ash. Nousiainen *et al.,* (2009).

Growth energy digestibility GED (g/kg) = −11.3 ± 14.78 + 0.977 ± 0.021× DOM. Where, DOM mean digestible organic matter.

Growth energy digestibility GED (g/kg) = −12.7 ± 18.4 + 1.00 ± 0.027× DDM. Where, DDM mean digestible dry matter.

TDN was calculated from ME value as per the equation of NRC (1989).

TDN (%) = [ME (MCal / kg DM) +0.45]/ 0.0445309

ME (MCal / kg DM) = ME (MJ/kg DM)/4.184

3. Milk measurements

After one week of postpartum, does were milked twice a day at 7.00 AM and 7.00 PM. The milk yield was recorded every week, kids were kept away from their mothers from 9 PM to 9 AM before each milking in a special and closed pen, and they were offered milk replacer and starter in order to obtain accurate values for daily milk yield. Milk samples were collected from all the animals of each experimental group, once every 25 days throughout the experimental period to determine its chemical composition. Moisture and ash content of milk was determined according to (AOAC, 2000). Total fat content was determined by Gerber test according to (Van den Berg, 1988). Lactose content was determined using method O'Mahoney (1988). goat's fat corrected milk was calculated according to equation of Mavrogenis and Papachristoforou (1988):

FCM (4%) kg = M x (0.411 + 0.147 x fat %). Where: M is milk production in kg.

3.1. Fractionation of milk fatty acid

Milk Fatty acids were determined by Gas Chromatography technique (GC) according to Aura *et al*. (1995). First, was fat extracted according to American Oil Chemists Society "AOAC" (2000) method. fatty acids were extracted and isolated according to the AOCS (1993) method. The extracted fatty acids were diluted in anhydrous diethyl ether (0.5-1.0ml) and methylated by adding diazomethane solution drop by drop until the yellow color was maintained (Vogel, 1975).

4. Blood measurements

After 2 months from postpartum, about 5 ml of blood were collected 4 h after feeding from the jugular vein of each experimental doe into a dry and clean tubes without anticoagulant. The collected blood was centrifuged at 3000 rpm for 30 min to get blood serum. Serum was collected into dried Eppendorf and frozen at −20°C. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to Reitman and Frankel (1975), cholesterol was measured according to Rolschlau (1974), triglycerides concentrations were quantified spectrophotometrically according to Fossati and Prencipe (1982) Serum total protein was measured according to the method of Armstrong and Carr (1964) and albumin was estimated according to Doumas et al. (1971). Globulin was calculated by subtracting the albumin from total protein. Serum Urea was determined by enzymatic colourimetric, urease salycilate method according to Patton and Crouch (1979) using the commercial kits from Sentinel CH. Creatinine determined method was applied according to the technique recommended by Julian (2000), Antioxidant capacities estimation was determined as Glutathione Peroxidase "GPx" and presence of Malondialdehyde (MDA) according to Fang *et*

al., (2011**) and** Wang *et al.,* (2011) respectively. The immunoglobulin G (IgG) and M (IgM) levels were estimated in the prepared goat serum using commercial bio diagnostic kits provided from Bio diagnostic Company (Giza, Egypt) and a spectrophotometer (Shimadzu, Japan) following the instruction directions.

5. Preparation of nanoemulsion oils

The formulation for oil-water emulsion consisted of 15% oil, 79.4% deionized water and 5.6% Tween 80 as the surfactant (Kentish *et al.,* 2008). Nanoemulsion was prepared by using an ultrasonic HIELSCHER UP50H processor for 20 minutes (El-Sherbiny *et al.,* 2016). A premixed emulsion was agitated at a frequency of 20 kHz, to decrease the size to nanodroplets. The formed emulsion pushed through a high shear area, resulting in uniformly sized droplets. This technique uses a water jacket to regulate the temperature. During ultrasonic emulsification, sonotrodes, also known as sonicator probes, used piezoelectric quartz crystals as energy sources. These sonotrodes shrink and expand when alternating electric voltage is applied. When the sonicator tip contacts the liquid, mechanical vibrations are produced which causes the vapor cavities inside the liquid to collapse producing nanodroplets in this liquid.

6. Characterization of nanoemulsion oil

6.1. Microscopie observations

The emulsion morphology and structure were investigated using a transmission electron microscopy (TEM) TOPCON 002B with a point-to point resolution of 0.18 nm and a voltage of 200 kV. The form and size of the emulsion, as well as the amorphous or crystalline character of their constituents, were revealed using a combination of bright field imaging at increasing magnification and diffraction modes. The concentrated emulsion was first diluted in water (1/10), then a drop of the diluted emulsion was directly put on the holey film grid and inspected after drying. The emulsion appears dark, while the surroundings appear bright, resulting in a positive image (figure 1). The direct observation also allowed us to examine the crystallinity of the emulsion core components using selected area electron diffraction (Singh and Vingkar (2008)).

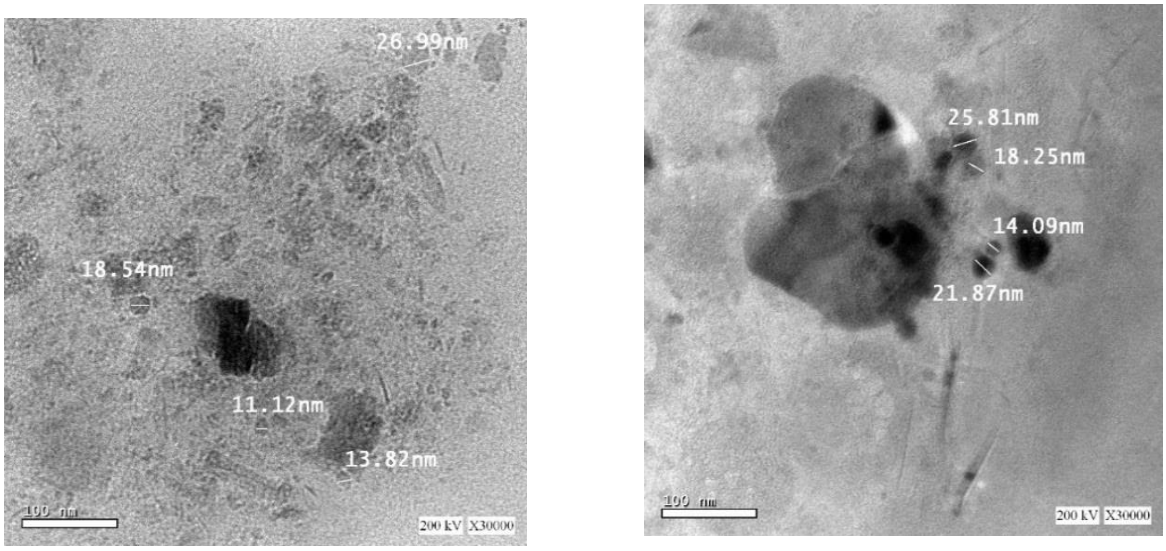

Figure (1). Transmission electron microscopic images of Nano emulsified essential oils blend (oregano, garlic, and clove essential oils).

6.2. Zeta potential

The zeta potential is used to determine surface charge of particles when it is submerged in liquid. The value of the zeta potential, which is used to forecast dispersion stability, is determined by the physicochemical properties of the emulsion, as well as the presence of electrolytes and their adsorption. The Malvern Zetasizer instrument is used to determining zeta potential. Nano emulsion is diluted to determine zeta potential, which is calculated based on the electrophoretic mobility of oil droplets. A zeta potential of ±30 mV is enough for ensuring nano emulsion physical stability (Đorđević *et al.,* 2015).

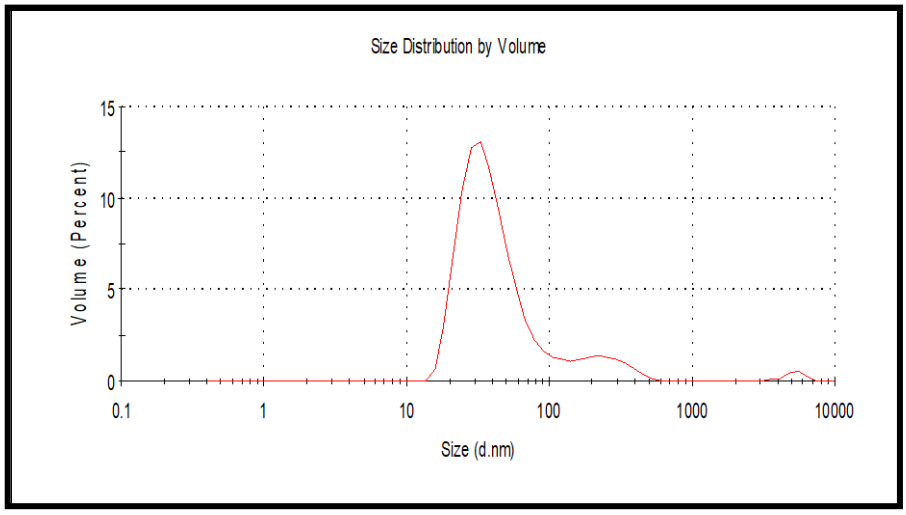

Figure (2). Zeta potentialof nano-emulsified esseintal oil

6.3. Emulsion stability index test

According to Meybodi et al. (2017), physical stability of emulsions was estimated by considering the amount of gravitational phase separation. Freshly formed nano emulsions (20 ml) (HE) were transported to cylindrical glass tubes (internal diameter 10 mm, height 50 mm) for physical stability testing, carefully closed, and stored at 25°C regulated incubator. The height of the translucent layer developed at the bottom of the emulsions (HS) was measured every week for 90 days to check of gravitational phase separation in glass tubes holding emulsions. The monitoring tests were done in triplicate, and the average of the three individual trials was used for data analysis in Table (3). The Equation used to calculate the emulsion stability index is: (ESI): ESI % = HE – HS / HE * 100

Table (3). Emulsion stability index test

DATE	Temperature	HS	HE	ESI %
Week 1	25	0 ml	20 ml	100
Week 2	25	0 ml	20 ml	100
Week 3	25	0 ml	20 ml	100
Week 4	25	0 ml	20 ml	100
Week 5	25	0 ml	20 ml	100
Week 6	25	0 ml	20 ml	100
Week 7	25	0.5 ml	20 ml	97.50
Week 8	25	1 ml	20 ml	95
Week 9	25	1 ml	20 ml	95
Week 10	25	1.5 ml	20 ml	92.50
Week 11	25	2 ml	20 ml	90
Week 12	25	2.5 ml	20 ml	87.50

Statistical analysis

The data collected from the experiment were analyzed using SAS (2013). Then, the comparison of means was done according to Duncan's (1955) multiple tests.

The linear model procedure (one-way ANOVA) was: $Yij = \mu + Ti + eij$,Where: Yij, μ, Ti and eij were Individual observation, overall mean, effect of treatment and random error, respectively.

RESULTS AND DISCUSSION

Effect of nano-emulsified essential oils blend on In vitro rumen gas production and rumen fermentation parameters:

Supplementation of NEOB to experimental groups reduced the GP (ml/200mg DM), GPSF (ml/g DM), GPSNF (ml/g DM) and SCFA (mml/ml gas) (P < 0. 05) when compared with the control group (Table 4). The highest (P<0.05) value of DDM, DOM, DCPL (g/day), DOMI (g/day), Microbial protein, GED (g/Kg OMD), GED (g/Kg DMD) and TDN were recorded for groups supplemented with NEOB. These results may be due to the supplementation effect of nano emulsified oil blend from essential oil that contains bioactive compounds which have been confirmed to modify ruminal fermentation by increasing the efficiency utilization of energy, while, decreasing rumen gas production especially methane production. Also, Essential oils have been play an important role as a potential modifier of ruminal biohydrogenation of dietary lipids, with the goal of producing healthy milk. Many researches emphasized that using essential oils riches of mono- unsaturated or poly-unsaturated fatty acids (PUFAs) had a positive impact of rumen gas production reduction. the efficiency essential oils on rumen methanogenesis depends on the concentration, type and fatty acid composition of essential oils (Beauchemin *et al.,* 2008). Essential oils rich in UFA used as a methane mitigation strategy. Essential oil not only increase dietary gross energy , but also, decrease rumen methane emissions by several reasons, including the decreasing in organic matter fermentation, rumen ciliate protozoa numbers, activity of methanogenic bacteria and use of hydrogen produced during biohydrogenation process by methanogenic bacteria to produce CH4 (Beauchemin *et al.,* 2009). Dietary lipids containing high levels of PUFAs for ruminants has been promising to reduce CH4 emissions from the ruminants according to the results of several studies (Martin *et al.,* 2010, Liu *et al.,* 2011 and Woodward *et al.,* 2006)

Table (4). Effect of nano-emulsified essential oils blend on *In vitro* rumen gas production and fermentation parameters in different experimental groups.

Parameter	Experimental group[1]			
	T1	T2	T3	SE**
GP (ml/200mg DM)	51.45^{a}	48.93^{b}	48.66^{b}	0.51
GPSF (ml/g DM)	13.97^{a}	10.45^{b}	10.22^{b}	0.17
GPNSF (ml/g DM)	47.48^{a}	30.15^{b}	24.68^{c}	0.37
SCFA (mml/ml gas)	114.39^{a}	107.72^{b}	107.80^{b}	0.47
DDM %	58.64^{c}	64.62^{b}	74.44^{a}	0.21
DOM %	67.63^{b}	70.60^{a}	72.05^{a}	0.08
ME (MJ/Kg DM)	9.03	9.43	9.44	0.24
NEL (MJ/Kg DM)	5.31^{b}	5.89^{a}	5.44^{ab}	0.15
NE	3.61^{b}	3.65^{b}	3.84^{a}	0.04
DCPL (g/day)	96.95^{c}	115.92^{b}	272.41^{a}	0.49
DOMI (g/day)	880.32^{c}	1022.24^{b}	1999.97^{a}	5.36
Microbial protein (g/DOM kg)	84.37^{c}	85.17^{a}	86.92^{a}	0.14
GED (g/Kg OMD)	57.15^{c}	57.68^{b}	59.05^{a}	0.11
GED (g/Kg DMD)	45.94^{c}	51.93^{b}	61.81^{a}	0.20
TDN (%)	58.63^{b}	62.95^{a}	62.78^{a}	0.27

[1] *T1, T2 and T3 refer to the experimental treatments*

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

Gas production (GP), Gas production structure fibre (GPSF) (ml/g DM), Gas production non-structure fibre (GPNSF) (ml/g DM). Digestible dry matter (DDM), Digestible organic matter (DOM), digestible organic matter intake (DOMI), digestible crude protein lactation (DCPL), metabolizable energy (ME`), Net energy (NE), net energy lactation (NEL), short chain fatty acid (SCFA).

The obtained results were agreed with, (Rui *et al.,* 2020) who reported that supplementing oregano essential oil (OEO) by different rates were investigated their effects on enhancing ruminal *in vitro*

fermentation parameters and decreasing total gas especially methane production. Liu *et al.*, 2017 reported that supplementing Oregano EO led to reduced ruminal methane production and positively improved apparent dietary digestible nutrients. (Dey *et al.*, 2021) found that supplementation of garlic oil reduced the enteric methane production by *in vitro* rumen fermentation trail from buffalo (Bubalus bubalis).

Patra and Yu (2012) reported that the *in vitro* tested of clove essential oil (CLO) and garlic essential oil (GAO) at three different doses (0.25, 0.50, and 1.0 g/liter), had significantly reduced methane production by increasing doses, with reductions by 34.4% and 87%, respectively, at 1.0 g/liter, also, two EOs significantly decreased of rumen archaea, protozoa, and major rumen cellulolytic bacteria (i.e., *Fibrobacter succinogenes*, *Ruminococcus flavefaciens*, and *R. albus*) linearly by increasing the doses of EOs.

Effect on lactating Trial:

Milk yield and feed efficiency:

The results of Tables (5) and (6) showed that there were significant differences (P<0.05) among the three tested groups in the milk production during the experimental period starting from week 2 to week 11 of the experiment. Where the highest(P<0.05) milk yield was observed for animals of group (T3) which fed on 7.5 ml NEOB daily, followed by those of (T2), while the control group (T1) had the lowest milk yield, starting from week 3 to the end of lactation period.

Table (5). Milk production at different stages of dairy Goats on different experimental groups.

Item	Milk production (g) (day)			
	T1	T2	T3	±SE
Week 2	129.6^{a}	123.2^{b}	100.8^{c}	1.07
Week 3	178.8^{b}	184.0^{b}	212.2^{a}	1.76
Week 4	297.6^{c}	318.4^{b}	519.6^{a}	1.43
Week 5	441.6^{c}	515.2^{b}	710.8^{a}	1.92
Week 6	568^{c}	667.2^{b}	900.8a	2.30
Week 7	737.6^{c}	836.8^{b}	1078.4^{a}	2.60
Week 8	820.8^{c}	940.8^{b}	1190.6^{a}	3.19
Week 9	822.4^{c}	937.6^{b}	1184^{a}	1.72
Week 10	569.6^{c}	619.2^{b}	908.8^{a}	3.15
Week 11	296^{c}	412.8^{b}	604.8^{a}	2.71

T1, T2 and T3 refer to the experimental ration
a,b,c, mean within some rows with differing superscript are significantly differ (P<0.05).

Table (6). Average daily feed intake, milk yield and Feed conversion ratio of the experimental does.

Item	Experimental ration			
	T1	T2	T3	±S E
Average daily feed intake kg (as feed) g/h/d				
Concentrate feed mixture	434.9^{c}	463.8^{b}	543.1^{a}	0.11
Clover hay	521.1^{c}	445^{b}	521.1^{a}	0.21
Average daily feed intake (on DM basis) kg/h/d				
Total DMI	0.853^{c}	0.909^{b}	1.064^{a}	0.20
Milk yield (kg/ h/ d)				
Actual daily milk yield	0.486^{b}	0.556^{b}	0.751^{a}	0.026
4% fat corrected milk	0.44^{b}	0.51^{b}	0.73^{a}	0.024
Feed conversion ratio				
DM kg / kg 4% FCM	1.94^{b}	1.76^{b}	1.45^{a}	0.029

T1, T2 and T3 refer to the experimental ration. a,b,c, mean within some rows with differing superscript are significantly differ (P<0.05).

groups fed diets supplemented with NEOB recorded significantly (P<0.05) higher of actual daily milk yield and 4% fat corrected milk compared with the control group. group supplemented with 7.5 ml NEOB/animal/day was significantly recorded (P<0.05) better values than control group in the term of feed conversion ratio (FCR) DM kg / kg 4% fat corrected milk (FCM). The improved in milk production as resulted of supplementing NEOB could be attributed to its antimicrobial effectiveness, a commonly has a potential activity of EOs is inhibiting methanogenic archaea which responsible for rumen gas production especially methane. Reducing methane emissions from ruminants led to reduce a loss of gross energy from feed. Thus, the reduction in methane formation from ruminal fermentation may increase feed efficiency and milk production (Vargas *et al.,* 2020 and Günal *et al*., 2017), Al-Suwaiegh et al. (2020) found the improvements in milk yield in early lactating Holstein dairy cows and feed efficiency when diet supplementing with (2.5 g/head/day) of EOs blend of clove, oregano, and juniper in equal proportions . They attributed these effects due to the inhibition of the rumen microbial populations by this dose of EOs blend. Few studies reported that feed intake was increased with the inclusion of EO blend (Kung *et al.,* 2008). El-Essawy *et al*. (2021) found that organic matter, ether extract and acid detergent fiber digestibility were higher (P < 0.05), as effected by supplementing essential oil (EOs) from anise oil , clove oil and thyme oil blend on lactating shami goats diet at daily dose of 2 mL/head/d. These results were not agreed with El-Essawy et al. (2021) Supplementations of EOs from anise oil, clove oil and thyme oil blend had no effect on milk yield (g/d).

Milk composition:

As shown in Table 7, 8 and 9. The results of the chemical composition of milk were showen on the 25, 50 and 75 days of the milk production period , group T3 supplemented with 7.5 ml NEOB/animal/day had recorded higher significant (P<0.05) differences comparing with control group in protein, fat, TS, Ash, and lactose content. The improved in milk composition as resulted of supplementing NEOB could be attributed to maintain of the gross energy losing by gas formation, that lead to provide higher net energy for milk production and increases the fat % specially UFA (El-Sherbiny *et al.,* 2016). In nano emulsion, tiny EO droplets are enhance their bioavailability (Nehme *et al.,* 2021).

Table (7). Milk composition of the experimental groups after 25 days of lactation

Item	Experimental rations			
	T1	T2	T3	±S
Protein	4.50^{b}	4.64^{ab}	4.78^{a}	0.043
Fat	3.40^{b}	3.54^{ab}	3.88^{a}	0.022
TS	12.84^{b}	13.30^{ab}	13.84^{a}	0.363
Lactose	3.18^{b}	3.28^{ab}	3.42^{a}	0.061
Ash	0.92^{b}	0.98^{ab}	1.08^{a}	0.007
Moisture	87.16^{a}	86.70^{ab}	86.16^{b}	0.363

T1, T2 and T3 refer to the experimental ration.

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

Table (8). Milk composition of the experimental groups after 50 day of lactation.

Item	Experimental ration			
	T1	T2	T3	±SE
Protein	4.56^{b}	4.68^{ab}	4.86^{a}	0.042
Fat	3.52^{b}	3.76^{ab}	4.18^{a}	0.100
TS	13.38^{b}	13.84^{ab}	14.36a	0.381
Lactose	3.24^{b}	3.30^{ab}	3.48^{a}	0.025
Ash	0.98^{b}	1.04^{ab}	1.12^{a}	0.007
Moisture	86.62^{a}	86.16^{ab}	85.64^{b}	0.381

T1, T2 and T3 refer to the experimental ration.

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

Table (9). Milk composition of the experimental groups after 75 days of lactation

Item	Experimental ration			
	T1	T2	T3	±SE
Protein	4.58[b]	4.76[ab]	5.00[a]	0.040
Fat	3.64[b]	3.94[ab]	4.34[a]	0.096
TS	13.72[b]	14.28[ab]	14.76[a]	0.334
Lactose	3.30	3.44	3.58	0.043
Ash	1.02[b]	1.10[ab]	1.18[a]	0.009
Moisture	86.28	85.72[ab]	85.24[b]	0.334

T1, T2 and T3 refer to the experimental ration.
a,b,c, mean within some rows with differing superscript are significantly differ (P<0.05).

Also, Nehme *et al. (*2021) emphasized that supplementing of EOs promote animal health and performance. (Lei *et al.,* 2019) reported that there are multiple effects of dietary supplement with EO on rumen microbiota, EOs could make better feed efficiency and nutrient utilization by ruminants. EO can also decrease protein degradation in the rumen. (Ratika and James Singh ,2018)). EOs may also influence the rumen degradation and metabolism of proteins by reducing the deamination reactions of amino acids, thus lower rumen ammonia production. In this meaning, EOs could prevent the increase of ammonia-producing bacteria, responsible about these reactions. Also, El-Essawy *et al.* (2021) found that fat yield (g/d) and fat content (g/kg) were greater (P < 0.05) with supplemented 2 mL/head/d of EOs blend of (anise oil (AO), clove oil (CO) and thyme oil (TO)) in lactating shami goats diet.

Milk fatty acids profile:

Results of milk fatty acid fractionation presented in Table (10). showed that animals of the control group (T1) recoded significantly (P<0.05) higher values of milk saturated fatty acids (SFAs) (Butyric acid (C4:0), Caproic acid (C6:0), Caprylic acid (C8:0), Capric acid (C10:0), Lauric acid (C12:0), Myristic acid (C14:0), Palmitic acid (C16:0) and Stearic acid (C18:0)) than those of T3 and T2.

Opposite results were optained for milk mono-unsaturated fatty acids (MUFAs) (Myristoleic acid (C14:1), Palmitoleic acid (C16:1) and Oleic acid (C18:1)) where values of T2 and T3 recorded (P<0.05) higher significant values compared with T1. Regarding results of poly-unsaturated fatty acids (Docosahexaenoic "DHA" (C22:6), Linolenic acid (C18:3), Eicosadienoic acid (C20:2), Dihomo-γ-linolenic acid (C20:3), Arachidonic acid (C20:4), Eicosapentaenoic acid "EPA" (C20:5), Docosapentaenoic "DPA" (C22:5), Linoleic acid (C18:2), animals of T3 and T2 had higher(P<0.05) significant values for these fatty acids, compared with the control group T1. The positive increase in UFAs (especially n-3 and n-6) was observed when nano emulsified oil blend form was supplemented which offered nanoscale fatty acid droplets, which led to increase the bioavailability of PUFA and could be effective in preserving higher proportions of PUFA in the rumen, which passes through the small intestine to the milk as an end product. Nano emulsified oil has a positive effect on reducing the rate of transformation of polyunsaturated fatty acids into saturated fatty acids in the bio-hydrogenation environment in the rumen, without affecting the total bacterial or protozoan count, reduces methane emission from dairy cows , increases the proportion of unsaturated fatty acids specially conjugated linoleic acid (CLA) that pass to the small intestine, increases the net energy for milk production and increases the milk fat% specially USFAs. UFA offers various potential health benefits, primarily attributed to CLA isomers and to fatty acids n-3 and n-6. Increasing the human diet's supply of UFA will help prevent or postpone atherosclerosis, coronary heart disease and helping to prevent inflammatory conditions. Increasing the supply of UFA can also slowing the growth of tumor cells.

The results reported herein, were agreed with those of many previous studies, Researchers around the world have established awareness that the amount of UFA, PUFA, or CLA in animal products mainly influenced by nutritional factor. The inclusion of UFA from the oil seeds and their impact on the FA profile in meat and milk was well documented (Irawan, *et al.,* 2017).

Butyrivibrio fibrisolvens, a bacteria that causes biohydrogenation, protozoa, and methanogen, will be suppressed by nano emulsified oils resulting in increased trans-11 (C18:1) (vaccenic acid) and conjugated linoleic acid (CLA) accumulation in the rumen , which reduce methane emission from animals (Irawan, *et al.,* 2017). Khiaosa-ard *et al.,* (2010) explained the mode of action of nano emulsified oils to reduce biohydrogenation pattern in the rumen and their effects on total bacteria, they indicated that the nanoscale diameter of the oil blend droplets inhibited bacterial chemical behavior while having no effect on the ruminal

bacteria's cellular structure. There is a reverse relationship between the USFA proportion in the milk and methane produced by the ruminant animals (Van Lingen *et al.*, 2014). El-Essawy *et al.*, (2021) found that composition of unsaturated, monounsaturated FA and polyunsaturated FA was higher ($P < 0.05$) with EO (anise oil (AO), clove oil (CO) and thyme oil (TO) blend) supplementations compared with control. Proportions of C18:1 n-9 C18:3 n-3 and n-6 FA were increased ($P < 0.05$) in goats fed EO compared to the control group. (El-Sherbiny *et al.*, 2016) reported that supplementing of nano emulsified oils increased significantly ($P < 0.001$) the proportions of oleic, linoleic and linolenic acids. Morsy *et al.*, (2012) found that supplementing EOs to dairy goat's diet affected of the quality of milk fat, by inhibiting bacteria which have the ability for bio-hydrogenation of unsaturated fatty acid. It was also confirmed that supplementation in anise, nails, clove, and juniper EOs improves the concentration of milk Conjugated Linoleic Acids (CLA) and omega 3 fatty acid.

Table (10). Fractionation of milk fatty acids of the experimental groups.

Item		Experimental group[1]			
	Item	T1	T2	T3	±SE
Saturated fatty acids (SFAs)	Butyric acid (C4:0)	2.20^{a}	2.00^{ab}	1.90^{b}	0.04
	Caproic acid (C6:0)	2.10^{a}	1.80^{b}	1.80^{b}	0.03
	Caprylic acid (C8:0)	3.40^{a}	2.60^{c}	3.00^{b}	0.03
	Capric acid (C10:0)	9.60^{a}	9.30^{b}	8.50^{c}	0.04
	Lauric acid (C12:0)	7.22^{a}	6.60^{b}	5.40^{c}	0.06
	Myristic acid (C14:0)	12.80^{a}	12.30^{b}	11.60^{c}	0.11
	Palmitic acid (C16:0)	28.90^{a}	26.80^{b}	25.90^{c}	0.06
	Stearic (C18:0)	8.42^{a}	7.70^{b}	7.20^{c}	0.06
Mono-Unsaturated fatty acids (MUFAs)	Myristoleic acid (C14:1)	0.30^{b}	0.40^{a}	0.40^{a}	0.01
	Palmitoleic acid (C16:1)	0.60^{c}	0.90^{b}	1.10^{a}	0.02
	Oleic acid (C18:1)	17.90^{c}	20.20^{b}	21.50^{a}	0.14
Poly-Unsaturated fatty acids (PUFAs)	Linoleic acid (C18:2)	1.90^{b}	2.30^{a}	2.50^{a}	0.05
	α-Linolenic acid (C18:3)	0.80^{c}	1.04^{b}	1.30^{a}	0.02
	Arachidonic acid (C20:4)	0.12^{b}	0.18^{a}	0.20^{a}	0.01
	Eicosapentaenoic acid (C20:5)	0.12^{b}	0.16^{ab}	0.22^{a}	0.01
	Docosapentaenoic acid (C22:5)	0.20^{b}	0.30^{a}	0.32^{a}	0.01

[1]T1, T2 and T3 refer to the experimental ration.
a,b,c, mean within some rows with differing superscript are significantly differ (P<0.05).

Blood constituents:

Results of blood analysis (Table 11) revealed that group T3 recorded ($P<0.05$) the highest albumin and Globulin values followed in a decreasing order by T2 and T1 respectively. Control group T1 was found to have ($P<0.05$) higher AST, ALT, cholesterol, triglycerides, urea and creatinine, while, the lower values were recorded with T3 respectively. all recorded results of blood chemical parameters for the three tested groups were found to be within the normal range of goat's blood analysis results (Samira *et al.*, 2016).

These results were agreed with El-Essawy *et al.*, (2021) founded a decrease (P = 0.05) in blood urea nitrogen (BUN) with lactating shami goats fed diet supplemented with EO blend of (anise oil, clove oil and thyme oil blend), while, other blood chemical parameters were not affected. On the other hand, immunity values in term of IgG and IgM found to be (P<0.05) higher in T3 compared with T2 and control (T1). the highest (P<0.05) significant blood antioxidant were recorded with T3 in term of GPx (U/mg protein) compared with T1, while, MDA parameter showed higher (P<0.05) significant differences with T1 and the lowest was recorded with T3 group.

Table (11). Animal blood constituents of different experimental groups

Item	Experimental ration			
	T1	T2	T3	±SE
AST (U/L)	77.64^{a}	74.7^{b}	72.66^{c}	0.690
ALT (U/L)	28.6^{a}	25.28^{b}	23.96^{c}	0.546
Cholesterol (g/dl)	82.14^{a}	75.26^{b}	70.7^{c}	1.000
Triglyceride (g/dl)	59.06^{a}	53.15^{b}	48.46^{c}	0.751
Total Protein (g/dl)	6.02^{b}	6.86^{a}	7.32^{a}	0.139
Albumin (g/dl)	4.06^{c}	4.74^{b}	5.12^{a}	0.064
Globulin (g/dl)	1.96^{b}	2.12^{ab}	2.2^{a}	0.018
Urea (g/dl)	23.64^{a}	22^{b}	20.44^{c}	0.385
Creatine (g/dl)	0.89^{a}	0.776^{b}	0.652^{c}	0.001
GPX (U/mg)	29.04^{c}	34.66^{b}	37.44^{a}	0.213
MDA (nmol/Ml)	3.02^{a}	2.42^{b}	1.98^{c}	0.015
IgG (mg/dl)	31.7^{c}	37.9^{b}	41.6^{a}	1.448
IgM (mg/dl)	53.84^{c}	60.04^{b}	62.5^{a}	1.237

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

Economic efficiency:

Results in Table 12. of the economic study showed that (T3) group was the highest daily feed cost (4.26LE) while the lowest daily feed cost (3.419LE) was observed for dairy goats fed control group (T1). The highest relative economic efficiency was significantly (P<0.05) recorded by (T3) being 176.89% when compared with the control group (100%). The better economic efficiency as a result of supplementation of NEOB could be regarding to the recorded increased in the milk yield and dairy goats' productive performance of this study.

Table (12): Effect of experimental rations on economic efficiency.

Item	Experimental rations			
	T1	T2	T3	**±SE**
Actual daily milk yield kg/h/day	0.486^{b}	0.556^{b}	0.751^{a}	0.026
Fat corrected milk (4%)	0.44^{b}	0.51^{b}	0.73^{a}	0.024
Total DMI	0.853^{c}	0.909^{b}	1.064^{a}	0.20
Daily feed cost (LE)	3.419^{c}	3.64^{b}	4.26^{a}	0.02
Av. Revenue daily of milk yield (LE)	5.47^{b}	6.26b	8.45^{a}	0.30
Net feed revenue (LE)	2.05^{b}	2.61^{b}	4.18^{a}	0.29
Economic feed efficiency %	60.15^{c}	71.85^{b}	98.14^{a}	0.77
Relative Economic efficiency %	100	137.76^{b}	176.89^{a}	2.96

T1, T2 and T3 refer to (control diet), control diet supplemented with 5 ml NEOB/ animal/day and 7.5 ml NEOB/animal/day, respectively. Market price at the time of experimentation for 1 ton CFM were 4500 LE/ ton Egyptian clover hay were 3500 LE / ton, price of 1kg goats' milk were 11.25 LE.

CONCLUSION

The nano emulsified oil blend form had beneficial effects on the efficiency of rumen fermentation parameters, reduction of total rumen gas production, increase the digestible dry matter and organic matter, production of healthy milk riches in total proportion of UFAs especially n-3 and n-6 and had low total proportion of SFAs, improvement milk yield , milk composition, significant increase in immunity values, and better blood antioxidant . Overall, these results support the recommendation of supplementation lactating shami goats' diet with NEOB (oregano, garlic, and clove essential oils) at 7.5 ml NEOB /head/day.

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تأثير مزيج زيت الأوريجانو والثوم والقرنفل المستحلب نانويا على مقاييس الكرش معمليا و الأداء الإنتاجي للماعز الشامي الحلابه.

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تم إجراء تجربتين لتقييم تأثير إضافة مستويات مختلفة من مزيج الزيوت العطرية النانوية المستحلب (NEOB) (زيت الأوريجانو والثوم والقرنفل) علي إنتاج الغاز الكلي بالكرش و مقاييس تخمر الكرش معمليا و علي أداء الماعز الحلابه . كانت التجربة الأولى عبارة عن تجربة تأثير الإضافة على إنتاج الغاز الكلي بالكرش ومقاييس تخمر الكرش في المختبر ، بينما كانت التجربة الثانية عبارة عن تجربه تأثير الإضافة على أداء الماعز الحلابه، تم تقسيم خمسة عشره ماعز شامي حلاب إلى ثلاث مجموعات متشابهة (5 حيوانات لكل مجموعة). كانت المجموعة التجريبية الأولى (T1) المجموعة الكنترول تتغذي (50% مخلوط العلف المركز : 50% ماده خشنه) ، بينما المجموعة الثانية (T2) تغذت على العليقة الكنترول مضاف اليها 5 مل من مزيج الزيوت العطرية النانوية المستحلب NEOB / رأس / يوم و أيضا تغذت المجموعة الثالثة (T3) على العليقة الكنترول مضاف اليها 7.5 مل NEOB / رأس / يوم. أظهرت النتائج أن المجموعات التجريبية المحتويه علي NEOB أظهرت انخفاضًا في إنتاج الغاز نتيجة الهضم المعملى الكلي في المختبر GP (ml / 200mg DM)، GPSF (ml / g DM)، GPSNF (ml / g DM) و ($p < 0.05$) SCFA (mml / ml gas) عند مقارنتها مع مجموعة الكنترول. كما أظهرت أيضا حيوانات هذه المجموعة (T3) أرتفاعا معنويا ($P < 0.05$) في إنتاج اللبن، محتوى بروتين اللبن ، الدهون ، TS ، الرماد ، اللاكتوز ، اللبن اليومي الفعلي و الدهون المعدله نسبه الدهن (4٪ دهن)، تليها المجموعة (T2). بينما سجلت المجموعة الكنترول (T1) أقل إنتاجية للبن من هذه المكونات مقارنا بجميع المجموعات. أيضا سجلت نتائج معدل التحويل الغذائي (FCR) من حيث DM كجم / كجم 4٪ (FCM) أن المجموعة المضاف لها 7.5 مل NEOB / حيوان / يوم أعطت قيمًا أفضل ($P < 0.05$) من مجموعة الكنترول. سجلت المجموعة T1 معنوياً ($P < 0.05$) أعلى إجمالي للأحماض الدهنية المشبعة (SFAs) ، بينما سجلت T2 و T3 قيمًا معنوية أعلى ($P < 0.05$) لإجمالي الأحماض الدهنية المتعددة غير المشبعة .((C22:6), (C18:3), (C20:2), (C20:3), (C20:4), (C20:5), (C22:5), (C18:2)). كما سجلت المجموعه المختبره T3 ($P < 0.05$) أعلى نسبة من الألبيومين والجلوبيولين ، وقيم المناعة من حيث (IgG و IgM) و مضادات الأكسدة GPx (U / mg protein) متبوعة بترتيب تنازلي بالمجموعه T2 و T1 على التوالي.