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Potentiality of oregano essential oils as a growth modulator, immune enhancer and natural antioxidative in mite infested Newzealand white rabbits

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A B S T R A C T

A total of twenty four unsexed New Zealand White (NZW) rabbits, weaned at 5 weeks of age, were observed to assess the impacts of some environmental stressors like stocking density (SD) and external parasite infestation (mite) on the growth performance and some blood biochemical parameters of this growing rabbits and assess the use of Oregano Essential Oil (OEO) supplementation as an antistressor for the rabbit intensification and mite infestation problems. NZW rabbits were housed in wire cages (45 cm x 45 cm x35 cm) in groups of 2 rabbits cage-1 (low stocking density; LSD) and 4 rabbits cage-1 (high stocking density; HSD). For the two densities, NZW rabbits were fed three diets with variable OEO levels and experimentally infested with P. cuniculi for 7 weeks by the following manner, (T1) group was the control group that has 0.0 OEO level kg-1 diet without mite infestations, (T2) and (T3) group were supplemented with 200 mg OEO kg-1 diet but only (T3) group was experimentally infested with mites, while the last treatment (T4) was experimentally infested with mites and supplemented with 400 mg OEO kg-1 diet. Finally, the current study found that a low dose of OEO-based feed had a favorable influence on the growth performance of NZW rabbits kept at LSD, with apparent benefits on antioxidant defense and innate immunity status. Our results also revealed that feed conversion ratio in rabbits provided with phytogenic supplemented diets had significantly lower values than in rabbits fed other treatments, indicating that phytogenics can promote growth. As a result, the practical consequences of phytogenic inclusion in rabbit feeds include the potential to improve rabbit growth performance and feed utilization. This would allow for faster rabbit growth, which would result in increased production time at optimal density while avoiding rabbits' parasitic infestations with mite. In conclusion, the findings of this study revealed that adding OEO in a dose of 200 mg kg-1 diet enhanced feed utilisation, rabbit somatic growth and ameliorating intensification and mite infestation stress effects on NZW rabbits.

Keywords: New Zealand White rabbit, Oregano essential oil, Stocking density, Mite infestations

1. Introduction

The increase of rabbit production, especially in the farming sector, is based on advances in exploitation techniques. Increased stocking density is an option that combines maximum food utilisation with increased rabbit production, but it may cause stress in the rabbits (Bhattacharjya et al., 2017). Rabbits are extremely susceptible to stressors such as overcrowding, temperature and mite infection. In any livestock enterprise, a high stocking density decreases production costs; however, an excessive density may negatively impact animal performance. As a result, knowing the optimal stocking density without affecting various production parameters is critical to increasing profitability (Bhattacharjya et al., 2017). In addition, a stressful environment suppresses the immune system, making rabbits more susceptible to infectious diseases and parasitic infestations; that common in rabbit farms resulting in significant financial losses.

Animal acariasis is a parasitic infection that affects animals' body surfaces or epidermis. Mites cause this essential ectoparasitic disease. It is a serious veterinary skin disease that decreases animal product yield and quality, also it is often fatal (O'Brien, 1999). Psoroptes mites are ectoparasites that infest a wide range of mammalian hosts. Ear mange is caused by the ear mite "Psoroptes cuniculi ", a highly contagious mite that mostly affects rabbits around the world. It lives within the ear pinnae, where it reproduces in a quick life cycle (Burke, 1992).

The use of Antibiotic Growth Promoters (AGPs) in animal feed for the promotion of growth and disease prevention has been prohibited by the World Health Organization (WHO) and many countries (Maron et al., 2013). Massive use of antimicrobials and vaccination for disease control has suppressed the growth in rabbit farms due to spread of antimicrobial-resistant parasites and the presence of antimicrobial residues in rabbit products and the environment (O'Brien, 1999; Gould, 2010). One of the most promising methods of controlling diseases in rabbit sectors are by strengthening the defence mechanism of rabbits through prophylactic administration of natural plant products (Elagawany, 2015) which is considered as a promising alternative to chemotherapy and vaccines (Secombes, 1994). One that has generated great interest among researchers is the introduction of oregano essential (OEO) oil in diets. OEO was recently verified to possess a valuable range of active substances; the most important are p-cymene, carvacol and thymol. The addition of essential oregano oil to rabbit feeds may promote wellbeing of rabbits and decreasing stress-related pitfalls induced by intensification practices. Oregano also is one of the most commercially valuable phytogenic additives in the world.

Finally, selecting an efficient phytogenic additive, that increases productivity while alleviating high density stress, reducing rabbit susceptibility to the serious problem of mite infestation and combating it, is a critical question for all veterinarians and rabbit owners. So, the aim of this study was to see how stocking density and mite infestation affected White New Zealand rabbits, and how Oregano Essential Oil (OEO) could modulate their effects**.**

2. Materials and Methods

2.1. Rabbit and experimental protocol

A. Experimental design and experimental rabbit rearing:

We used a weaned New Zealand White (NZW) developing rabbits of both sexes, who were weaned at 5 weeks of age and had an initial body weight of 600±40 g. Sakha Animal Prod. Res. Inst. Station (SAPIS) in Kafr El-Sheikh Province, Egypt, provided the experimental rabbits. Four treatment groups of two rabbits for low stocking density (LSD) and four rabbits for high stocking density (HSD) were randomly distributed (table 1). The rabbits were placed in the rearing cages for a week of adaptation. They were fed an experimental diet (Supper Rabbit® Feed) as a basal diet. During the 56-days experiment (with the adaptation week), rabbits were held in equal management, hygienic, and environmental conditions. The rabbits were handled according to the principles for the care of laboratory animals (Lebas et al., 1986) during the study, and the experiment was approved by the Animal Husbandry and Animal Wealth Development Department's Committee Ethics.

B. Diet preparation: during the whole experiment, a commercial diet for growing rabbits were fed to them according to (Trocino et al., 2008).

Also, 100 g molasses kg-1 diet is added to the basal ration as carrier for Ropadiar 10%®, where individual ration batches were made by adding the exact amount of molasses and oregano essential oil to 3 kg batches of the basal ration to be fed as freshly prepared diets to rabbits.

2.2. Performance calculations and proximate analysis

A. Growth measurements: Growth performance parameters, body final weight (FW), average weight gain (AWG), average daily gain (ADG) and feed conversion ratio (FCR), of NZW growing rabbits were calculated according to (Botsoglou et al., 2004).

B. Haematological parameters and immune responses of different groups under experiment:

1. Blood Collection:

samples were taken at random from the rabbit ear veins. To avoid hairs mixing with the blood, rabbit ears were wiped and cleaned. The blood was collected from the rabbits through the ear vein with a 3 ml plastic syringe without injuring the rabbits. Hematological analysis was performed after 1.5 mL of blood was transferred to Ethylene Diamine Tetra Acetic Acid (EDTA) tubes. On the day of collection, haematological tests on EDTA samples were completed. The other 1.5 mL of blood was moved into biochemistry test tubes. After the blood was coagulated, the tubes were centrifuged at 3500 rpm for 15 minutes to separate the serum, which was then kept below -200C for biochemical analysis.

2. Haematological analysis: all the following haematological parameters were performed according to the method described by (Grindem, 2011).

A. Total red blood corpuscles (RBCS) and total leukocytic counts (WBCS) as well as, the differential counts of leucocytes (lymphocyte and neutrophil).

B. Haemoglobin content (Hb) and platelets (PLT).

C. Packed cell volume (PCV %), Mean Corpuscular Haemoglobin (MCH) (g) = Hb X 10 / Red blood cell and Mean Corpuscular Haemoglobin Concentration (MCHC) (g dl-1) = Hb X 100 / Packed cell volume.

2.3. Immunological analysis

A. Serum biochemical analyses:

1. The serum total protein was calculated according to the Biuret method using a commercial kit (Spectrum kits, Biodiagnostic Industry, Co), while the albumin value was determined using the bromocresol green method (Richard et al., 1974). The globulin was calculated by subtracting plasma albumin from total protein and albumin-globulin ratio (A/G) was also calculated.

2. Biochemical indices including the free plasma levels of glucose and cholesterol were measured according to (Bakr et al., 2017).

3.The levels of serum cortisol were measured using commercial kits (Spectrum kits, Biodiagnostic Industry, Co), following the method described by manufacturer.

B. Liver and kidney function tests in serum:

1. According to (Reitman and Frankel, 1957), the serum enzyme activities of transaminases in blood, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured.

2. Serum urea nitrogen and creatinine levels were also determined according to the methods described by (Patton and Crouch, 1977) using a commercial kits (Spinreact kits, Sigma Industry, Co).

C. Assay of serum Glutathione Peroxidase (GPx) enzyme activity:

The evaluation based on the methods described in the instructions, using a commercial kit (Spinreact kits, Sigma Industry, Co).

3. Experimental mite infestation:

Four rabbits, with a median age of six months and a history of alopecia and crusting for 20 days, were brought to our experimental site from a neighboring farm where they had been infected by a mange outbreak. Mange lesions with itchy skin, painful ears, erythematous areas due to pruritus on the ears and crust formation, necrosis and gryposis on toes were discovered on physical examination. Mange was suspected based on the clinical symptoms and characteristics of the lesions. Microscopic examination of skin scrapings was done for confirmation. To remove debris on the slide and facilitate inspection by light microscopy under low power magnification (x10) skin scrapings were prepared with a 10% solution of sodium hydroxide or mineral oil (Voyvoda et al., 2005). On microscopic examination of these skin scrapings, Psoroptes cuniculi at all developmental stages – egg, larva, nymph, and adult – were described.

P. cuniculi was also identified, with mobility and morphology that matched those reported in a previous study by (Bowman, 2009). After housing our White New Zealand rabbits for one week, we conducted an experimental infestation by putting the four rabbits in T3 and T4 cages for two weeks. P. cuniculi infestation was discovered in the rabbits of these two treatments during the pretreatment assessment. Both rabbits were examined under a microscope. On microscopic examination of ear swabs of the external ear canal combined with sodium hydroxide or mineral oil, viable mites were also observed. Erythema, crusts, and sensitivity on palpation of the ears were some of the clinical symptoms of P. cuniculi infection in New Zealand rabbits.

4. Statistical analysis:

The data were statistically processed using GLM procedure for one-way analysis of variance (SAS, 2002), based on a linear model to measure all data effects.

3. Results and discussion

OEO supplementation can help rabbits perform better not only because of better nutrient utilization in their diets, but also because of other nutritional factors like improved nutrient transportation and absorption, lipid and energy metabolism, and antioxidant activity among others (Zeng et al. (2015). Generally, higher stocking density and rabbit mite infestations had a negative effect on the growth performance characteristics of the growing rabbits. In agreement with the current findings, (Aboegla et al., 2013; Sherif et al., 2019) who discovered that rabbits stocked at 2 rabbits cage-1 had significantly higher total feed intake (FI), daily weight gain, and final live weights than those stocked at 3 and 4 rabbits cage-1. However, contrasting findings were reported by (Paci et al., 2013; Volek et al., 2014), in which stocking density had no effect on rabbit growth performance parameters.

Also, in our study, the FW, TAWG, TADG and TFCR of mite-infested rabbit groups (MIRG) reared at LSD or HSD but fed a diet supplemented with 200 mg OEO kg-1 were significantly better than those of other MIRG. In the same way, (Seddiek et al., 2013) found that the infested and non-treated groups' growth performance parameters were significantly lower than the CAN (aqueous leaf extract of neem) treated groups'. So, rearing of NZW rabbits at LSD with dietary inclusion of 200 mg kg-1 OEO in their diets, not only alleviated the drastic effects of HSD and mite infestations of NZW rabbits, but also significantly improved their growth performance parameters. However, as the amount of oregano in the feed increased, FI and feed conversion efficiency both decreased linearly. Increasing OEO concentration in the diets may have given the rabbits a bad taste. Thus, the lower FI in rabbits fed the highest oregano inclusion rate (400 mg kg-1 diet) may have been due to an unpalatable diet, as the flavor of the diet has been shown to promote or decrease FI in animals (Deyoe et al., 1962).

Table (2): The effect of different stocking density, addition of oregano essential oil (OEO) and mite infestation on the final weight (FW), total average weight gain (TAWG), total average daily gain (TADG), (g rabbit-1), and total feed conversion ratio (TFCR, %) of NZW rabbits.

According to (Rotolo et al., 2013), the growth performances of animals fed diets supplemented with aromatic plants like OEO were higher than those of animals in the control group, with the OEO supplemented group achieving the highest final body weight. Our findings are also in consistent with those of (Dalle Zotte et al., 2016). On the other hand, (Botsoglou et al., 2004; Soultos et al., 2009) found that dietary oregano essential oil addition at concentrations of 0, 100, or 200 mg kg-1 diet had no significant effects on all rabbit performance parameters.

Changes in haematological parameters can be used to evaluate the effect of nutritional factors and additives supplied in the diet on any living creature, so that (Svobodova et al., 1991) proposed haematology as a useful matter in determining feed composition and nutritional status in relation to rabbit environmental conditions. Our current experimental results showed that all blood pictures had significant alteration under different stocking densities and mite infestations. The high WBCs count observed in this study in case of increased stocking density and/or mite infestation of rabbits may be reflective of stress, which elicits a defensive response.

In agreement with the current report (Aboegla et al., 2013; El-Bayoumi et al., 2018). Also, there are significantly decrease of neutrophils (%) and significantly increase of lymphocytes (%) when increasing stocking density as indicated by (Beshara et al., 2019). The addition of 200 mg Ropadiar 10%® kg-1 to WNZ rabbits' diets, improved all haematological parameters of these rabbits, particularly those reared under LSD. Accordingly, (Hekal et al., 2016) explaining the promising positive results

of OEO on the haematological parameters but (Szabóová et al., 2013) has a contrary findings. These results indicated that OEO-fed rabbits are healthy, with an effective immune status and are none anemic animals.

The nutritional, physiological and immunological status of experimentally growing rabbits could be calculated by using serum blood protein parameters (Bakr et al., 2017). Our results showed improved serum TP and Glob levels in the experimental rabbit groups reared at LSD and fed diet containing Ropadiar 10%® 200 mg kg-1, with the none MIRG having higher levels than those mite-infested. However, increasing the SD led to insignificant increase of Alb and A/G ratio especially in MIRG. Similar negative effects of SD have been identified by (Aboegla et al., 2013; El-Bayoumi et al., 2018). But, in contrasts to the findings described here, (Dorra et al., 2013; Sherif, 2018) suggesting that rabbit SD had no effect on plasma levels of TP, Alb or Glob. This indicated that the improved rabbits TP and Glob may be linked to the supplementation of commercial and natural OEO, suggesting an improvement in the diet's nutritional values, growth performance, physiological functions and health status of these rabbits in the face of mite infestation and high stocking density stresses. These results are confirmed by (Ashour et al., 2014) on mice and pigs and (Abdallah et al., 2020) on rabbits.

Chronic stress may disturb the normal physiology of vital organs such as liver and kidneys. Stressors like mite infestation and rabbit intensification affect hepatic and renal cells by inducing the formation of toxic products (free radicals), which destroy hepatocytes and renal cells. These effects were reflected through a significant intensification in the serum ALT, AST, Urea-nitrogen and Creatinine levels of the mite infested rabbit groups reared at high stocking densities. In partial agreement with that, (Aboegla et al., 2013) found that AST activity was higher in rabbits stocked in groups with 3 and 4 rabbits cage-1 than in the others, whereas ALT activity was unaffected by the cage density. Similarly, (El-Bayoumi et al., 2018) found that HSD (28 rabbit m-2) had negative effects on stress-related parameters such as serum creatinine, which displayed the highest levels when compared to rabbits housed at 20 and 12 rabbits m-2. The unchanged values of liver and kidney enzymes than reference ranges for healthy rabbits in normal rabbits fed an OEO 200 mg kg-1 diet, suggesting that no damage to the liver and kidney occurred in that groups. Similar positive effects of OEO addition on the health status of rabbit livers were previously discovered by (Abdallah et al., 2020). Also, in partial agreement with the present work, rabbits fed 1.5 % and 3 % marjoram had lower blood serum creatinine, according to (Bakr et al., 2017). However, (Omer et al., 2013) found that OEO had no significant impact on creatinine levels. So, rearing of rabbits at low stocking density with dietary inclusion of OEO in their diets, improved their liver and kidney health.

Increased cage density resulted in a decrease in cholesterol levels, which may suggest a general decrease in lipid mobilisation in the other groups, in parallel with this (Kalaba, 2012; Aboegla et al., 2013) discovered that rabbits housed at the HSD had significantly lower plasma cholesterol levels than those housed at LSD. A controversy results were reported by (El-Bayoumi et al., 2018; Sherif, 2018; Sherif et al., 2019) who found that cage density had no effect on serum cholesterol levels in the growing rabbits. The OEO addition decreases serum Chol levels either at LSD or HSD. It also appeared to be lower with 200 mg kg-1 OEO than 400 mg kg-1 OEO supplemented diet. Moreover, MIRG fed 400 mg kg-1 OEO supplemented diet has higher serum Chol values than other MIRG fed 200 mg kg-1 OEO supplemented diet. These findings matched those of (Hong et al., 2012; Omer et al., 2013) who suggested that adding oregano as feed additives to rabbit diets significantly lowered total Chol levels. This was supported by (Crowell, 1999) who discovered that the pure components of essential oils inhibit Chol synthesis by inhibiting the production of hepatic 3-hydroxy-3-methy glutaryl coenzyme A (HMG-CoA) reductase, which is a key regulatory enzyme in Chol synthesis(Lee, 2002).

The physiological changes of rabbits are a significant factor in determining their general health, unsuitable environmental conditions or the presence of stress and stressors like intensive culture and mite infestations. Although our results revealed that there were no significant differences in glucose and cortisol (especially cortisol) concentrations, there was a trend toward higher concentrations in rabbits housed at HSD and infested with mites. This was explained previously by (Summers, 2006) who cleared that as the stress load increases, the demand for blood glucose increases, adrenalin is released, and glycogen body reserves are mobilized, resulting in low blood glucose levels. In a complete agreement with our results, (Kalaba, 2012; El-Bayoumi et al., 2018) found that plasma glucose and cortisol levels were apparently higher in rabbits housed at the maximum density than those housed at LSD. A contrary studies by(Buijs et al., 2009) showed that stocking density had no effect on the physiological stress indicators.

Here, glucose and cortisol levels of the experimental rabbits fed Ropadiar 10%® 200 mg kg-1 diet and held at LSD decreased significantly compared with the other treatments and also, stocking rabbits at HSD with dietary inclusion of OEO in their diets, improved rabbit responses to the stressful conditions of HSD and mite infestations. Similarly, (Ghazi et al., 2015) using vitamin C and OEO supplementation to diet, revealed that serum glucose concentrations decreased. Also in partial agreement with the current research, (Bakr et al., 2017) approved that feeding rabbits 1.5% rosemary or marjoram decreased serum glucose significantly. Although serum glucose has often been considered to be a useful nonspecific stress indicator, the lower glucose levels in our study may be due to the hypoglycaemic effects of carvacrol (a main compartment of OEO) (Deng et al., 2013).

In our study, glutathione peroxidase (GPx) activity was strongly affected by the stocking density, as the cellular antioxidant levels were decreased by increasing the stocking density of rabbits. Moreover, the activities and values of the antioxidant enzyme (GPx) was assessed and markedly enhanced in the plasma of rabbits fed diet containing Ropadiar 10%® 200 mg kg-1 compared to other treatments.

So, rearing of rabbits at LSD with dietary inclusion of OEO in their diets, improved their antioxidant enzymes activity in terms of glutathione peroxidase (GPx). Typically, (Botsoglou et al., 2004), found that dietary supplementation with 200 mg OEO kg-1 diet had a major antioxidant impact in rabbits, providing indirect evidence that antioxidant compounds in OEO are absorbed by the rabbit gut, thus increasing tissue antioxidant activity (Cardinali et al., 2015). However, a recent study by (Rotolo et al., 2013) found that no differences in oxidative stability of rabbits fattened on a diet supplemented with oregano or sage dry leaves at a 1% dietary inclusion level, but this variation may be due to the low concentration of the active compounds. Some common phytogenic products, such as OEO, in vitro antioxidant activity was found to be well associated with their total phenolic content (Yang et al., 2002). The presence of OH phenolic groups, which donate hydrogen to the proxy radicals formed during the first step in lipid oxidation, thus retarding the formation of hydroxyl peroxide, has been suggested as an explanation for OEO's antioxidant activity. The phytogenic, which primarily includes 3 OH-groups and can supply H atoms to quench free radicals, had the highest total polyphenolic content and flavonoids (Jeon et al., 2001).

4. Conclusion

This study declares the impact of stocking density and mite infestation stressors and the role of OEO on modulating these stressors on NZW rabbits. NZW rabbits reared under high stocking densities and infested with mite had reduced performance parameters, impaired haemato-biochemical indices, decreased antioxidative and immunity responses; however, the addition oregano essential oils to their diets ameliorated the performance, biohealth status, antioxidant properties and immunity of NZW rabbits reared under the two rearing facilities. Hence, the findings of this study revealed that adding essential oregano oil to rabbit diets improved feed utilisation and antioxidant parameters. It also shows that phytogenic additives can influence innate immunity parameters. As a result, it's possible that oregano's dietary usage will increase in the future as a result of positive appreciation of consumers is anticipated, as well as its effectiveness.

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Table (1): The experimental design:

Treatment	Stocking density		Oregano essential oil kg^{-1} (OEO)	Mite infestation
	Type	No. of rabbit $cage^{-1}$		
T_1	Low (LSD)	2 (10 rabbits m^{-2})	Control (0)	Not infested
	High (HSD)	4 (20 rabbits m^{-2})	Control (0)	Not infested
T_2	Low (LSD)	2 (10 rabbits m^{-2})	200 mg	Not infested
	High (HSD)	4 (20 rabbits m^{-2})	200 mg	Not infested
T_3	Low (LSD)	2 (10 rabbits m^{-2})	200 mg	Infested
	High (HSD)	4 (20 rabbits m^{-2})	200 mg	Infested
T_4	Low (LSD)	2 (10 rabbits m^{-2})	400 mg	Infested
	High (HSD)	4 (20 rabbits m^{-2})	400 mg	Infested

Table (2): The effect of different stocking density, addition of oregano essential oil (OEO) and mite infestation on the final weight (FW), total average weight gain (TAWG), total average daily gain (TADG), (g rabbit-1), and total feed conversion ratio (TFCR, %) of NZW rabbits.

Item		FW	TAWG	TADG	TFCR
T_1 (Control)	LSD (2 Rabbit $Cage^{-1}$)	2460 ± 10^{bc}	1825 ± 0^{bc}	241.43 ± 10.71^{ab}	3.15 ± 0^{cd}
	HSD (4 Rabbit $Cage^{-1}$)	2192.5 ± 72.87^{e}	1557.5 ± 76.66^{e}	210.71 ± 8.63^{cd}	3.75 ± 0.13^{ab}
T_2 (200 mg OEO Kg^{-1} diet)	LSD (2 Rabbit $Cage^{-1}$)	2652.5 ± 22.5^{a}	2012.5 ± 17.5^{a}	254.29 ± 0.71^{a}	2.76 ± 0.12^{d}
	HSD (4 Rabbit $Cage^{-1}$)	2348.75 ± 42.44^{cde}	1708.75 ± 32.62^{cde}	224.29 ± 5.98^{bcd}	3.26 ± 0.03^{bcd}
T_3 (200 mg OEO Kg^{-1} diet, with mite infestation)	LSD (2 Rabbit $Cage^{-1}$)	2587 ± 12^{ab}	1947 ± 23^{ab}	253.5 ± 2.79^{a}	2.87 ± 0.11^{cd}
	HSD (4 Rabbit $Cage^{-1}$)	2292.5 ± 74.32^{cde}	1658.75 ± 72.67^{cde}	221.79 ± 10.64^{bcd}	3.33 ± 0.2^{bc}
T_4 (400 mg OEO Kg^{-1} diet, with mite infestation)	LSD (2 Rabbit $Cage^{-1}$)	2402.5 ± 17.5^{bcd}	1760 ± 0^{cd}	237.86 ± 10.71^{abc}	3.21 ± 0.05^{cd}
	HSD (4 Rabbit $Cage^{-1}$)	2218.75 ± 30.71^{de}	1581.25 ± 40.38^{de}	208.57 ± 4.32^{d}	3.77 ± 0.18^{ab}

Table (3): The effect of stocking density, oregano essential oil (OEO) and mite infestation on haematological RBCs parameters of NZW rabbits.

Item		Hb (mg dl^{-1})	RBCs ($\times 10^6 \mu L^{-1}$)	PCV %	MCV (g)	MCH (g)	MCHC (g dl^{-1})
T_1 (Control)	LSD (2 Rabbit $Cage^{-1}$)	12.95 ±0.15 bc	4.6± 0.1 bc	48± 1 ab	104.35±0.09 ab	22.3 ±0.8 1 b	21.37±0.76 b
	HSD (4 Rabbit $Cage^{-1}$)	10.25 ±0.15 e	3.65 ±0.1 5 ef	36.5 ±2.5 c	100.45±1 0.98 b c	32.1 5±5. 57 ab	31.78±2.07 ab
T_2 (200 mg OEO Kg^{-1} diet)	LSD (2 Rabbit $Cage^{-1}$)	14.15 ±0.05 a	5.15 ±0.0 5 a	49.5 ±0.5 a	96.12±0.04 bc	26.2 ±1.1 ab	27.26±1.14 ab
	HSD (4 Rabbit $Cage^{-1}$)	11.05 ±0.15 cd	3.85 ±0.0 5 cde	46± 5 ab	119.33±1 1.44 a b	29.6 3±1. 68 ab	25.2 ±3.8 3 ab
T_3 (200 mg OEO Kg^{-1} diet, with mite infestation)	LSD (2 Rabbit $Cage^{-1}$)	13.55 ±0.35 ab	4.85 ±0.0 5 ab	47.5 ±0.5 ab	97.94±0.02 bc	28.0 3±0. 74 ab	28.62±0.75 ab
	HSD (4 Rabbit $Cage^{-1}$)	10.65 ±0.15 de	3.7± 0.1 de	34± 1 c	91.9 ±0.2 bc	28.3 ±1.7 ab	30.8 ±1.9 ab
T_4 (400 mg OEO Kg^{-1} diet, with mite infestation)	LSD (2 Rabbit $Cage^{-1}$)	12.65 ±0.15 bc	4.45 ±0.2 5 bc	45.5 ±3.5 ab	102.13±2.13 ab c	28.4 6±4. 63 ab	27.98±5.12 ab
	HSD (4 Rabbit $Cage^{-1}$)	10.2± 0.1 e	3.45 ±0.1 5 f	34.5 ±2.5 c	99.87±2.9 bc	31.6 2±7. 17 ab	31.89±8.11 ab

Table (4): The effect of stocking density, oregano essential oil (OEO) and mite infestation on haematological parameters of WBCs and platelets of NZW rabbits.

Item		WBCs	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	Platelets
T_1 (Control)	LSD (2 Rabbit $Cage^{-1}$)	4900 ±800 d	66.5 ±0.5 cde	23.5 ±1.5 d	2.5± 0.5 a	1.5± 0.5 a	251 ±6 d
	HSD (4 Rabbit $Cage^{-1}$)	5350 ±150 bcd	64± 3 e	25±1 bcd	2.5± 0.5 a	1±0 a b	309.5±4.5 bc
T_2 (200 mg OEO Kg^{-1} diet)	LSD (2 Rabbit $Cage^{-1}$)	5100 ±800 bcd	70± 0 abcd	26±1 abcd	2.5± 0.5 a	1±1 a b	265.5±1.5 cd
	HSD (4 Rabbit $Cage^{-1}$)	5500 ±600 bcd	67± 2 cde	30±1 ab	3±1 a	1±0 b	331.5±8.5 a
T_3 (200 mg OEO Kg^{-1} diet, with mite infestation)	LSD (2 Rabbit $Cage^{-1}$)	8050 ±1050 abc	73.5 ±2.5 a	29±1 abc	3±1 a	0±0 a b	259.5±2.5 d
	HSD (4 Rabbit $Cage^{-1}$)	8750 ±1450 a	71± 1 abc	31±1 a	3.5± 1.5 a	1±0 a b	313 ±2 bc
T_4 (400 mg OEO Kg^{-1} diet, with mite infestation)	LSD (2 Rabbit $Cage^{-1}$)	5000 ±100 c d	66.5 ±1.5 cde	24±3 cd	2.5± 0.5 a	1.5± 0.5 a	246.5±5.5 d
	HSD (4 Rabbit $Cage^{-1}$)	5350 ±550 bcd	65± 0 de	26±1 abcd	2.5± 0.5 a	1±0 a b	306 ±4 bc

Table (5): The effect of stocking density, oregano essential oil (OEO) and mite infestation on serum blood proteins of NZW rabbits.

Item		Total protein (g dl^{-1})	Albumin (g dl^{-1})	Globulin (g dl^{-1})	A/G ratio
T_1 (Control)	LSD (2 Rabbit $Cage^{-1}$)	7.6±0.3 bc	2.6±0.3 ab	5±0.6 bc	0.53± 0.12 ab
	HSD (4 Rabbit $Cage^{-1}$)	6.8±0 e	2.55± 0.15 ab	4.25± 0.15 c	0.6±0.06 ab
T_2 (200 mg OEO Kg^{-1} diet)	LSD (2 Rabbit $Cage^{-1}$)	8.15±0.05 a	2.85± 0.55 ab	5.3±0.5 abc	0.55± 0.16 ab
	HSD (4 Rabbit $Cage^{-1}$)	7.15±0.05 cde	2.25± 0.25 ab	4.9±0.3 bc	0.46± 0.08 ab
T_3 (200 mg OEO Kg^{-1} diet, with mite infestation)	LSD (2 Rabbit $Cage^{-1}$)	7.8±0.1 ab	1.5±0.2 b	6.3±0.1 a	0.24± 0.04 b
	HSD (4 Rabbit $Cage^{-1}$)	7±0.1 cde	2.95± 0.35 a	4.05± 0.25 c	0.74± 0.13 a
T_4 (400 mg OEO Kg^{-1} diet, with mite infestation)	LSD (2 Rabbit $Cage^{-1}$)	7.55±0.15 bc	2.9±0.2 a	4.65± 0.35 bc	0.63± 0.09 ab
	HSD (4 Rabbit $Cage^{-1}$)	6.8±0 e	2.8±0.1 a	4±0.1 c	0.7±0.04 a

Table (6): The effect of stocking density, oregano essential oil (OEO) and mite infestation on serum liver and kidney enzymes activities of NZW rabbits.

Item		Alanine aminotransferase (ALT)	Aspartate aminotransferase (AST)	Urea	Creatinine
T_1 (Control)	LSD (2 Rabbit Cage^{-1})	68.5±3.5^{e}	31±2^{c}	41.5±6.5^{d}	0.97±0.02^{b}
	HSD (4 Rabbit Cage^{-1})	94.5±5.5^{de}	36±4^{c}	45.5±5.5^{d}	1.15±0.25^{b}
T_2 (200 mg OEO Kg^{-1} diet)	LSD (2 Rabbit Cage^{-1})	59.5±2.5^{e}	25.5±2.5^{c}	33.5±5.5^{d}	0.93±0.02^{b}
	HSD (4 Rabbit Cage^{-1})	88.5±6.5^{de}	32.5±7.5^{c}	42.5±1.5^{d}	1.05±0.01^{b}
T_3 (200 mg OEO Kg^{-1} diet, with mite infestation)	LSD (2 Rabbit Cage^{-1})	121.5±6.5^{cd}	60.5±6.5^{b}	73.5±4.5^{c}	1.11±0.01^{b}
	HSD (4 Rabbit Cage^{-1})	144.5±14.5^{bc}	76.5±5.5^{ab}	80.5±9.5^{bc}	1.18±0.01^{ab}
T_4 (400 mg OEO Kg^{-1} diet, with mite infestation)	LSD (2 Rabbit Cage^{-1})	128±16^{cd}	62.5±4.5^{b}	88.5±3.5^{bc}	1.14±0.06^{b}
	HSD (4 Rabbit Cage^{-1})	177.5±17.5^{ab}	92±3^{a}	101±4^{a}	1.35±0.05^{a}

Table (7): The effect of stocking density, oregano essential oil (OEO) and mite infestation on the serum biochemical parameters (cholesterol (Chol), cortisol (Cort), glucose (Glu) and glutathione peroxidase (GPx) levels of NZW rabbits.

Item		Cholesterol (mg dl^{-1})	Glucose (mg dl^{-1})	Cortisol (ng dl^{-1})	GPx (UL^{-1})
T_1 (Control)	LSD (2 Rabbit Cage^{-1})	104.5±4.5^{bcd}	86.5±1.5^{d}	6.7±0.1^{b}	33.5±1.5^{de}
	HSD (4 Rabbit Cage^{-1})	87±10^{cde}	98.5±1.5^{cd}	8.2±0.9^{b}	28.5±0.5^{e}
T_2 (200 mg OEO Kg^{-1} diet)	LSD (2 Rabbit Cage^{-1})	56±3^{ef}	79.5±0.5^{d}	6.1±0.1^{b}	63.5±3.5^{a}
	HSD (4 Rabbit Cage^{-1})	47.5±1.5^{f}	90±1^{d}	7.25±0.15^{b}	49±0^{b}
T_3 (200 mg OEO Kg^{-1} diet, with mite infestation)	LSD (2 Rabbit Cage^{-1})	76.5±2.5^{def}	101.5±3.5^{bcd}	16.85±0.35^{a}	53.5±0.5^{b}
	HSD (4 Rabbit Cage^{-1})	68±1^{def}	126±13^{ab}	17.25±0.75^{a}	42±0^{c}
T_4 (400 mg OEO Kg^{-1} diet, with mite infestation)	LSD (2 Rabbit Cage^{-1})	124±30^{abc}	121.5±6.5^{abc}	16.85±2.35^{a}	51±1^{b}
	HSD (4 Rabbit Cage^{-1})	80.5±3.5^{def}	137.5±7.5^{a}	19.6±0.4^{a}	38±2^{cd}