

THE COMPLEMENTARY EFFECT BETWEEN GARLIC, CINNAMON AND JUNIPER ESSENTIAL OILS ON PRODUCTIVE PERFORMANCE, DIGESTIBILITY AND BLOOD PARAMETERS OF NEW-ZEALAND WHITE RABBITS

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SUMMARY

A total of 72 male New Zealand White (NZW) rabbits after weaning were allotted among 4 groups with 3 replications, six rabbits each, using a completely randomized design, for 45 d. The 1st group (control, R1) was fed a basal diet without addition. The 2nd, 3rd and 4th groups were fed a basal diet supplemented with 0.25 mg of garlic oil (GAR) plus 0.25 mg of cinnamon oil (CIN, R2); 0.25 mg of GAR plus 0.25 mg of juniper oil (JUN, R3) and 0.25 mg of GAR plus the mixture of CIN and JUN at the level of 0.125 mg for each / kg diet v/w (R4), respectively. The results indicated that dietary rabbit's GAR plus a mixture of CIN and JUN significantly increased the total bacterial count by 44.4% and the cellulolytic bacteria by 70%, respectively, compared to the control group. Dietary rabbit's GAR plus CIN (R2) and JUN (R3) significantly improved the DM digestibility by 41.7 and 29.1% as well as increased the EE digestibility by 18.7 and 13.9%, respectively compared to the control group. The mixture treatment (R4) significantly increased the final body weight by 11.9%, the total body weight gain by 7.5%, the average daily body weight gain by 51.5% and feed conversion ratio by 17.2% compared to the control group. The mixture treatment (R4) significantly decreased the triglycerides by 54.6%, total cholesterol by 67.5, LDL by 52.5% followed by (R3) treatment by 53, 32.4 and 79.2%, respectively. Same reductions were detected in (R2) treatment by 49.7, 33.2 and 30.9%, respectively compared to the control group. The mixture treatment (R4) significantly increased the carcass weight (with or without edible offals) by 11.9 or by 12.6%, respectively compared to the control group. In conclusion, dietary rabbit's GAR plus a mixture of CIN and JUN significantly increased caecum microbial count and further improved nutrients digestibility, growth performance, carcass characteristics complemented with decreasing serum lipids profile.

Keywords: *New Zealand White (NZW) rabbits, essential oils, caecum bacterial count, growth performance and carcass characteristics.*

INTRODUCTION

After preventing the use of antibiotics as growth stimuli, rising compression has begun on the livestock industry therefore, essential oils (EOs) are considered as a safe alternative, they are considered natural bioactive ingredients derived from plants and have positive effects on animal growth and health (Puvača *et al.*, 2014). The use of plant-based feed additives as a growth promoter has been documented in many studies as these additives play an active role in the reproductive and productive performance of domestic animals (Windisch *et al.*, 2008; Elagib *et al.*, 2013; Abdel-Wareth and Lohakare, 2014; Ashour *et al.*, 2014 and Li *et al.*, 2016). Global importance in herbal products has increased significantly with cows, sheep and goats accounting for the largest percentage followed by poultry and rabbits (31, 17, 17, 9.1 and 4.3%), respectively (Viegi *et al.*, 2003).

To enhance general health conditions in humans and animals, by-products of herbal supplements, such as EOs, saponins and tannins have become a major source of feed additives and antioxidants. Rabbits and poultry are good, fast and cheap sources of white meat. Therefore, in recent times efforts of many researchers have been directed to evaluate the use of herbal and plant secondary as appropriate feed additives suitable for their needs. Moreover, herbal feed additives have been found to improve the average daily gain (ADG) and feed conversion ratio (FCR), reduce mortality and increase the viability of rabbits (El-Kholy *et al.*, 2012 and Zeweil *et al.*, 2013). Several EOs, plant extracts and nutritional herbs have been studied for their ability to enhance growth in monogastric animals (Cross *et al.*, 2007). The utilization of herbal plants as nutritional supplements in rabbits and the evaluation of increased performance of rabbit diets to improve productive performance is a new direction in the study of livestock.

Garlic include a variety of organosulfur compounds as diallyl disulfide, allicin and ajoene (Chi *et al.*, 1982). Allicin is responsible for any activity as antibacterial (Cavallito *et al.*, 1944), as well as immune-enhancing activities (Kyo *et al.*, 1998). Cinnamon contains derivatives such as cinnamic acid, cinnamaldehyde and many other ingredients such as antioxidants, polyphenols, antimicrobial and anti-inflammatory effects that have beneficial effects against many diseases. (Hariri and Ghiasvand, 2016). Juniper contains: α - and β -pinenes, terpinene-4-ol, limonene as well as other pseudofructus compounds as flavonoids. Juniper has applicability in several medical applications (as antioxidant, antiviral and antibacterial agents), because due to their polyphenolic compounds content (Soukand *et al.*, 2015).

EOs of medicinal plants contains many ingredients that have the potential to play positively for gut microbes or cecum fermentation and to improve growth performance and quality of animal products (Simitzis, 2017). The EOs are hydrophobic substances that enter the pathological bacterial cell wall and disrupt the integrity of the cell membrane, which leads to an imbalance in the osmotic pressure of the bad cells and disrupts them from functioning (Calo *et al.*, 2015). The organosulfur compounds (OSC) are present mainly in garlic and onion that account for only 1% of garlic weight which much greater than those of garlic phenolic compounds (Lu *et al.*, 2017). The OSC exhibits an over 500-fold increase in antibacterial efficacy to kill several pathogenic and drug-resistant bacteria (Xu *et al.*, 2018) and antioxidant activity (Sagdic and Tornuk 2012).

The phenolic compounds (PhC) in most of the medicinal plants include phenolic acids, flavonoids, curcuminoids, coumarins and others are responsible for their chemo-preventive properties e.g., antioxidant or antimutagenic and anti-inflammatory effects (Cai *et al.*, 2004) as well as contribute apoptosis by arresting cell cycle, regulating metabolism (Huang *et al.*, 2010). Therefore, the PhC are often insufficient to protect from mutagens, which leads to the need for dietary supplementation as an alternative approach (Fresco *et al.*, 2006).

So, the study looks for the complementary effect between the organosulfur compounds in garlic oil (GAR) and the phenolic compounds in cinnamon oil (CIN) and juniper oil (JUN). This work aimed to study the complementary between GAR with either CIN and JUN EOs on rabbit's performance, digestibility coefficient, caecum microorganism's count, blood parameters and carcass characteristics.

MATERIALS AND METHODS

Experimental design and dietary treatment:

This work was carried out at Research and Production Station, National Research Centre located in El-Emam Malik Village, El-Bostan, West of Nubaria and at laboratories of Animal Production Department, Parasitology & Animal Diseases Department and Agricultural Microbiology Department, NRC and was designed to study the effect of supplementation of GAR, CIN and JUN in growing rabbit diets using seventy-two male NZW rabbits after weaning with an average body weight of 818 ± 37 g. Rabbits were housed in individual wire cages and divided into four equal treatment groups of 18 rabbits each three replicates of six each.

Feeding and management:

The basal experimental diet was formulated and pelleted to cover the nutrient requirements of rabbits as a basal diet according to NRC (1977) as shown in Table (1). This basal experimental diet was similar to that used by El-Nomeary *et al.* (2020). The feeding period was extended for 45 d. and the experimental groups were classified as follows: The 1st group (control, R1) was fed a basal diet without addition. The 2nd, 3rd and 4th groups were fed a basal diet supplemented with 0.25 mg of GAR plus 0.25 mg of CIN(R2);

0.25 mg of GAR plus 0.25 mg of JUN (R3) and 0.25 mg of GAR plus the mixture of CIN and JUN at the level of 0.125 mg for each/ kg diet v/w (R4), respectively. The GAR, CIN and JUN used in this study were sprayed by 0.5 ml /kg diet v/w on daily pelleted feed intake to avoid loss of some volatile oils and to ensure the effect of fresh oils for rabbits. Rabbits were individually housed in galvanized wire cages (30 x 35 x 40 cm). Stainless steel nipples for drinking and feeders allowing the recording of individual feed intake for each rabbit were supplied for each cage. Feed and water were offered *ad libitum*. Rabbits of all groups were kept under the same administrative conditions and were individually weighed. Feed consumption was individually recorded bi-weekly during the experimental period.

Essential oils supplementation:

The EOs used in the study was purchased from El-Captain Company (Cap- Pharm) for extracting natural oils, plants and cosmetics, License of Ministry of Health, No 337006. The garlic was extracted by the supercritical fluid extraction (SFE), cinnamon was extracted using steam distillation via separatory funnel and juniper was extracted by instantaneous controlled pressure drop (DIC) which allowed us to extract 95% of EOs.

Table (1): Composition and chemical analysis of the basal diet.

Ingredients	Content %
Clover hay	32
Yellow corn	22.00
Wheat bran	30.00
Soybean meal	14.00
Limestone	1.13
Vit.&min. mix*	0.30
Common salt	0.40
DL-methionine	0.17
Total	100.00
Chemical analysis (% on DM basis)	
Dry matter (DM)	92.81
Organic matter (OM)	90.89
Crude protein (CP)	16.50
Crude fiber (CF)	14.00
Ether extract (EE)	3.00
Ash	9.11
Nitrogen free extract (NFE)	57.39
Gross energy** (Kcal/Kg DM)	4176.93
Digestible energy ***(Kcal/Kg DM)	2481.12

R1: Control diet. R2: Control diet + 0.25mg garlic oil +0.25 mg cinnamon oil. R3: Control diet + 0.25 mg garlic oil + 0.25mg juniper oil. R4: Control diet + 0.25mg garlic oil + 0.125mg cinnamon oil plus 0.125mg juniper oil/ kg diet.

** Vit. & Min. mixture: Each kilogram of Vit. & Min. mixture contains: 2000.000 IU Vit. A, 150.000 IU Vit. D, 8.33 g Vit. E, 0.33 g Vit. K, 0.33 g Vit. B1, 1.0 g Vit. B2, 0.33g Vit. B6, 8.33 g Vit.B5, 1.7 mg Vit. B12, 3.33 g pantothenic acid, 33 mg biotin, 0.83g folic acid, 200 g choline chloride, 11.7 g Zn, 12.5 g Fe, 16.6 mg Se, 16.6 mg Co, 66.7 g Mg and 5 g Mn.*

*** Gross energy (GE) was calculated according to Blaxter (1968). Each g CP = 5.65 kcal, g EE = 9.40 kcal and g (CF & NFE) = 4.15 kcal.*

****Digestible energy (DE) was calculated according to Fekete and Gippert (1986) using the following equation: DE (kcal/ kg DM) = 4253 – 32.6 (CF %) – 144.4 (total ash %).*

All rabbits were used in digestibility trials over 7 d to determine the nutrients digestion coefficients and nutritive values of the tested diets. Feed intake of experimental rations and weight of feces were recorded daily. Representative samples of feces were dried at 60°C for 48 h, grinded and stored for chemical analysis later. Chemical analysis of the basal diet and feces were analyzed according to AOAC (2000) methods. Gross energy (GE) was calculated according to Blaxter (1968) and digestible energy (DE) was calculated according to Fekete and Gippert (1986).

Slaughter trial:

Five representative rabbits from each treatment group were chosen around treatment mean average BW fasted for 12 h before slaughtering according to Blasco *et al.* (1993) to determine the carcass

measurements. These were removed and individually weighed. Weights of edible and external offals were calculated as percentages of slaughter weight (SW). The hot carcass was weighed and divided into front, middle and hind parts.

Caecum microorganism's preparation:

Microbiological evaluation: the study was carried out using five rabbits per treatment group and was selected for sample collection concerning body weight compared to group mean body weight. The caecum content was placed on agar plates for analysis. The serial dilution plate count procedure was used to estimate the total number of different groups of micro-organisms. The most numbers of bacteria were obtained from the positive tubes using method of Hoskins (1934). Decomposition of the cellulose medium was used for aerobic cellulose decomposing organisms (Dubos, 1928). Total bacterial count, 10^5 and cellulolytic bacteria, 10^5 was according to Espina *et al.* (2016). The total number of caecum bacteria was counted according to Difco (1989). The technique of colony-forming unit (CFU) was adopted and incubation took place at 30°C for 2- 7 d.

Serum biochemical studies:

Before slaughtering of rabbits at the end of the experiment, blood samples were collected (5 rabbits /group) through vein puncture, placed in a plain centrifuge tube, and centrifuged at 2000 rpm for 15 minutes to separate clear serum which stored at -20°C until further biochemical analysis.

Serum proteins profile: Determination of total proteins was performed according to the method of Henry *et al.* (1974) and albumin (Doumas *et al.*, 1971), the used test kits were supplied by bio-Mérieux, France. Serum globulins were determined by subtracting the value of serum albumin from the value of serum total proteins, also the A/G ratio was calculated. Electrophoresis was performed using a Semi-automated agarose gel electrophoresis system (Helena Laboratories Helena Biosciences, Gateshead, UK) according to the manufacturer's instructions. Using the computer software Phoresis (Helena Biosciences), electrophoretic curves plus related quantitative specific protein concentrations for each sample were displayed. Relative protein concentrations within each fraction were determined as the optical absorbance percentage, and absolute concentrations (g/dL) were calculated using the total serum protein concentration, total cholesterol (Allain *et al.* 1974), triglycerides (Fossati and Prencipe 1982), activities of aminotransferases (GOT) (Reitman and Frankel 1957), urea according to Patton and Crouch (1977) and creatinine according to Husdan (1968). Commercial diagnostic kits from Biomerieux, France, were used for the assay of serum biochemical parameters.

Statistical analysis:

Collected data were subjected to statistical analysis as a one-way classification analysis of variance using the general linear model procedure of SPSS (1998). Duncan Multiple Range Test (Duncan,1955) was used to separate means when the dietary treatment effect was significant according to the following model: $Y_{ij} = \mu + T_i + e_{ij}$ Where:

Y_{ij} = observation. μ = overall mean.

T_i = effect of experimental rations for $i = 1-4$.

e_{ij} = the experimental error.

RESULTS AND DISCUSSION

Effect of using mixtures of essential oils in feed on rabbit's digestibility coefficient:

Data of Table (2) showed that ration groups adding mixture of EOs significantly ($P < 0.05$) enhanced both CP and ether extract digestibility, meanwhile, insignificantly improved for dry matter, organic matter, crude fiber and NFE in compare with the control group. The largest values of nutrient digestibility and nutritive values were realized occurred with rabbits received ration contained GAR plus CIN and JUN (R4). Groups fed GAR plus CIN (R2) significantly ($P < 0.05$) increased the EE by 18.7 and 13.9%, respectively compared to the control group (Table 2). These results probably because EOs are a mixture of substances that have the ability to stimulate the secretion (bile and saliva) and enhance the activity of enzymes such as trypsin and amylase in part by increasing the ability of epithelial tissues to increase the retention time of the stomach for feed, which leads to increase digestion and absorption (Platel and Srinivasan, 2004). Also, probably due to that the mixture of EOs able to return a significant protein that increments glutathione enzymes in the liver that conserve damaged cells and improve organ function

(Patra *et al.*, 2001 and Shehata *et al.*, 2003) or may be because the ability of some EOs to boost the S-transferase enzyme system as allylsulfides in garlic (Kyo *et al.*, 1998). In other words, or because of its characteristics as antimicrobial as in the rumen, or it may be that the mixture of EOs can manipulate the process of metabolism of ceacum and inhibit the formation of methanogenesis selectively (Boadi *et al.*, 2004).

The combination of EOs beneficial affects the ecosystem of the intestinal microflora by relieving oxidative stress induced by them, controlling potential pathogens and stabilizing intestinal microbes (Zeng *et al.*, 2015). The mixture EOs appears to suppress harmful microorganisms stimulates beneficial microbes such as *Lactobacillus spp.* The reason for the improvement in nutrient absorption may be partly explained due to stimulation in secretions of saliva, bile and enhanced enzyme activity (Platel and Srinivasan, 2004 and Jang *et al.*, 2007). Due to the wide variety of phytochemicals present in plant herbs, they can stimulate digestive secretions and regulate feed intake, but affect digestion processes differently (Frankič *et al.*, 2009). In a recent study, adding a compound (Digestaroms) containing a mixture of caraway, fennel, onion, garlic, cloves and anise to rabbit rations worsened the digestibility of cellulose and EE (Celia *et al.*, 2016). The beneficial effects of nutritional inclusion of phytochemicals on digestion of nutrients, gut health, growth performance and intestinal integrity have been reported earlier (Abdel-Wareth *et al.*, 2012 and Brenes and Roura, 2010). Besides, the improved use of feed with EOs in this study could be a result of the stimulating effect of EOs on the digestion operation, and these results indicate that enhanced digestion coefficient of nutritious leads to extra balanced intestinal microflora with the ability to decrease the ratio of harmful microorganisms as reported by Langhout (2000) and Williams and Losa (2001). Such as the results reported by Amad *et al.* (2011) who found that herbal feed additives enhanced the apparent digestibility of the nutrients at 21, 35 and 42 days of age. The results of crude protein and crude fiber digestibility agree with Patra *et al.* (2001) and Shehata *et al.* (2003), who indicated that the supplement of garlic with different levels increased ($P<0.05$) significantly CP and CF digestibility. The nutritive values of nutrients digestion of experimental treatments are shown in Table (2). The results showed that the addition of GAR plus a mixture of CIN (R2) significantly decreased the DCP compared to the control group. These results declared that the addition of garlic did not impact DCP, this result agrees with the result of Patra *et al.* (2001) and Shehata *et al.* (2003) who reported that the addition of garlic with different levels not improved DCP and not significantly ($P<0.05$) TDN.

Table (2): Nutrients digestibility for growing rabbits supplemented with different essential oils mixture.

Item	Experimental diets				±SE	Sig.
	R1 (control)	GAR oil 0.25mg/kg diet R2	R3	R4		
Body weight(kg)	2.31 ^a	1.64 ^b	1.75 ^b	2.18 ^{ab}	0.15	*
DM intake, g/h/d	143.24 ^a	83.53 ^c	101.57 ^{bc}	125.50 ^{ab}	8.69	*
DM intake, g/ kg BW	61.68	53.87	57.41	57.51	5.06	NS
Nutrients digestibility,%:						
DM	60.22	56.08	60.94	58.47	2.39	NS
OM	66.52	60.71	66.46	65.03	2.02	NS
CP	78.37 ^a	70.29 ^b	75.53 ^a	75.44 ^a	1.06	*
CF	33.08	25.77	32.08	29.77	4.02	NS
EE	68.72 ^b	81.59 ^a	78.31 ^a	71.00 ^b	1.62	*
NFE	74.03	68.17	74.50	73.29	1.99	NS
Nutritive value,%:						
TDN	57.17	52.93	57.55	56.08	1.69	NS
DCP	12.31 ^a	11.23 ^b	11.86 ^{ab}	11.84 ^{ab}	0.19	*

R1: Control diet. R2: Control diet + 0.25mg garlic oil +0.25 mg cinnamon oil. R3: Control diet + 0.25 mg garlic oil+ 0.25mg juniper oil. R4: Control diet + 0.25mg garlic oil + 0.125mg cinnamon oil plus 0.125mg juniper oil/ kg diet.

^{a,b and c}: means in the same row within each treatment having different superscripts differ significantly at $P<0.05$. SE: Standard error of the mean. NS: Non-significant. * $P<0.05$.

Effect of using mixtures of essential oils in feed on the rabbit’s growth performance:

As shown in Table (3) the results mentioned that addition GAR plus mixture of CIN + JUN in rabbit rations improved final weight, total BWG, ADG and better FCR. Meanwhile, it registered the second value of feed intake (FI) compared with the other groups. Dietary rabbit’s GAR plus mixture of CIN +

JUN significantly increased the final body weight by 7.5%, the total body weight gain by 11.9%, the ADG by 51.5%, as well as improved the feed conversion ratio by 17.2 %, respectively compared to the R1 group (Table 3). These outcomes probably due to that allyl sulfides in garlic enhance glutathione S-transferase enzyme system (Kyo *et al.*, 1998) and inhibit the growth of intestinal bacteria such as *Staphylococcus aureus* and *Escherichia coli* as well as inhibit aflatoxins producing fungi (Amagase *et al.*, 2001). Also, probably due to the effect of cinnamaldehyde in CIN that increased the DMI of feed in the early week of the fattening period similar as in cattle (Yang *et al.*, 2010) especially in small doses as well as may be due to the effect of JUN that used to treat diarrhoea and useful as an appetizer (Khan *et al.*, 2012).

In poultry, the combination of EOs of CIN, thyme, oregano and citrus fruits (Lippens *et al.*, 2005) or laurel, citrus and anise (Cabuk *et al.*, 2006) increased the rate of FCR. A mixture of EOs taken from wild-growing herbs in Turkey has been found to impact BW, FI, FCR and carcass properties when utilized as a feed additive for the broiler (Alcicek *et al.*, 2003 and 2004). Supplemental dietary GAR and mixture EOs in rabbit diets led to improve live body weight (LBW). This increase in live body weight with supplementation of EOs mixture may be due to the provision of some compounds that enhance absorption and digestion of certain nutrients in the diet, which may be attributed to the bioactive ingredients (allicin) present in garlic causing better effectiveness in the benefit of the diet, which resulted in promoting growth. Our results partially agree with Gbenga *et al.* (2009) who concluded that BWG, FI and FCR were not significantly influenced by dietary garlic supplementation; it was observed that the animals consuming a high concentration of garlic supplement had a slight increase in BWG compared to the animals fed the basic ration. This may be due to the increment amounts of protein available at the cellular level for deposit in the body tissues. This finding is harmonious with the reports of Ortsergu *et al.* (2008) and Ademola *et al.* (2005).

Feed intake of all groups was decreased with the added mixture of EOs in rabbit rations compared with the control group (R1). The additives mixture at 0.5 mg/ kg diet decreased DM feed intake, this assumption was supported that the high dose of the additive mixture contains a relatively high amount of phenolic compounds, which led to decrease in feed intake and digestion compared to the low dose. The present results agree with those declared by Morshedy *et al.* (2019), results indicated that a significant ($P \leq 0.01$) decrease in the feed intake was observed in the groups fed peppermint essential oil and essential oil blend compared to the control group. Kotsampasi *et al.* (2018) concluded that EOs at a high level of 450 mg/kg resulted in a lower FI compared to the low levels of 150 and 300 mg/kg in lambs. Our findings on FI and FC are also in present concur with those of Halle *et al.* (2004), who indicated that supplementation of oregano and its EOs reduced the daily FI of broilers and significantly improves FCR. The get better in nutrition efficiency achieved with mixtures of EOs can be cited for their enhance the impact at the digestibility of nutrients, as reported by Langhout (2000), Madrid *et al.* (2003) and Hernandez *et al.* (2004). Contrary to our finding, Lee *et al.* (2003), Botsoglu *et al.* (2004) and Hernandez *et al.* (2004) found that the supplement of herbal extracts or mixture EOs to the ration did not affect on FI or feed conversion ratio.

Table (3): Growth performance of growing rabbits supplemented with different essential oils mixture.

Item	Experimental diets				±SE	Sig.
	R1 (Control)	GAR oil 0.25mg/kg diet				
		R2	R3	R4		
Initial body weight, g	811.17	816.50	792.50	813.33	78.58	NS
Final body weight, g	2122.33 ^b	2130.66 ^b	2018.66 ^b	2280.66 ^a	75.63	*
Total body weight gain, g	1311.17 ^b	1314.16 ^b	1316.16 ^b	1467.33 ^a	53.07	*
Average daily body weight gain, g	29.14 ^b	29.20 ^b	29.24 ^b	32.60 ^a	0.86	*
Dry matter intake, g/h/d	105.00 ^a	67.35 ^c	88.37 ^b	96.11 ^{ab}	3.39	**
Feed conversion, kg DMI/kg gain	3.60 ^a	2.31 ^c	3.02 ^b	2.98 ^b	0.06	**

R1: Control diet. R2: Control diet + 0.25mg garlic oil +0.25 mg cinnamon oil. R3: Control diet + 0.25 mg garlic oil+ 0.25mg juniper oil. R4: Control diet + 0.25mg garlic oil + 0.125mg cinnamon oil plus 0.125mg juniper oil/ kg diet.

^{a,b and c:} means in the same row within each treatment having different superscripts differ significantly at $P < 0.05$.

SE: Standard error of the mean. NS: Non-significant. *: $P < 0.05$. **: $P < 0.01$.

Furthermore, Ibrahim *et al.* (2000) showed an increase ($P < 0.05$) in FC when feeding male weaned NZW rabbits with 0.5% herbaceous plants. They noted that the improvement in feed conversion with garlic probably due to improved feed efficiency and organ function. Javandel *et al.* (2008) also, adding medicine plants such as garlic and ginger as growth promoters in broiler ration and watched a clear improvement in their BWG and FCR. Furthermore, Jamroz *et al.* (2003) showed that by adding 150 or 300 mg/kg of a mixture of oils include carvacrol, capsaicin and cinnamaldehyde for the diet led to improve BW. In this study, the increase in BWG was harmonious with the findings reported by Hertrampf (2001); McCartney (2002); Alçiçek *et al.* (2003) and Dermer *et al.* (2003).

Effect of essential oils on rabbit's caecum microorganism's count:

Dietary rabbit's GAR plus the mixture of CIN and JUN (R4) significantly increased the total bacterial count by 44.4% and the cellulolytic bacteria by 70%, respectively compared to the control group (Table 4). In mixture EOs, the improvement of beneficial caecum microorganism's count may be attributed to the diallyl disulfide in garlic that increased the microbial population especially the total *E. coli* bacterial count (Hernandez *et al.*, 2004) and may be also due to the cinnamaldehyde in CIN that decreased the proportion of acetate producing bacteria which belong mostly to gram-positive bacteria (McIntosh *et al.*, 2003) which be considered as suitable feed additives to manipulate caecum microbial fermentation as showed in ruminant nutrition (Markey *et al.*, 2011). It's also, may be due to the hydrophobic nature of the antimicrobial components present in the JUN (Ramdani *et al.*, 2013) leading to increasing slowing down bacterial growth via inducing disturbances of the pathogenic bacteria cell osmotic pressure (Calo *et al.*, 2015).

The results showed that the addition of GAR plus a mixture of CIN and JUN (R4) significantly increased the count of cellulites bacteria compared to the control group. The antibacterial efficacy of garlic versus *S.typhi* and harmful organisms were discussed by Fleischauer and Arab (2001) to be linked to the presence of several ingredients such as diallyl sulfides, ajorene, alliin and organosulfur compounds, which make garlic a strong disease-battle factor. On the other hand, these results may be in general due to the effects of EOs components that have the potential to positively manipulate gut microbiota, caecum fermentation considering the beneficial effects of caecum microorganisms via stimulating *Lactobacillus spp.* and protect gut villi (Simitzis, 2017).

The like impact was noted through nutritional supplementation containing 0.5 g/ kg DM of thyme oil, which enhanced gut integrity and demonstrated a tendency to activate many beneficial microbes in the gut of rabbits (Placha *et al.*, 2013). The phenolic components of EOs possess antimicrobial efficacy against many microorganisms by changing the permeability of the cytoplasmic film to hydrogen and potassium ions, which mainly disrupt basic cellular processes (Costa *et al.*, 2013).

Table (4): Caecum microorganism's count of rabbits supplemented with different mixture essential oils.

Item	Experimental diets				±SE	Sig.
	R1	GAR oil 0.25mg/kg diet				
	(Control)	R2	R3	R4		
Total bacterial count,10 ⁵	45.00 ^d	62.00 ^b	55.67 ^c	65.00 ^a	3.25	*
Cellulolytic bacteria,10 ⁵	50.00 ^b	82.0 ^a	78.00 ^a	85.00 ^a	2.13	*

R1: Control diet. R2: Control diet + 0.25mg garlic oil +0.25 mg cinnamon oil. R3: Control diet + 0.25 mg garlic oil+ 0.25mg juniper oil. R4: Control diet + 0.25mg garlic oil + 0.125mg cinnamon oil plus 0.125mg juniper oil/ kg diet.

^{a,b,c and d}: means in the same row within each treatment having different superscripts differ significantly at $P < 0.05$.

SE: Standard error of the mean. *: $P < 0.05$.

Effect of using mixtures of essential oils on blood parameters of rabbits:

Dietary rabbit's GAR plus the mixture of CIN+JUN significantly increased the total protein by 6.42%, globulins by 19.2%, alpha globulins by 28.1% and gamma globulins by 65.7%, respectively compared to the control group (Table 5). These results probably due to the protection functions against oxidative stress (Su *et al.*, 2018). Dietary rabbit's GAR plus CIN significantly increased the total protein by 10.1%, globulins by 39.2%, alpha globulins by 37.5% and beta globulin by 30% and gamma globulin by 80%, respectively compared to the control group (Table 5).

The significant increase in total protein probably due to cinnamaldehyde successfully prevents the protein imbalance and causes of occurrence cell death by demonstrating the neuroprotective potential of these natural compounds in oxidative stress (Maiolo *et al.*, 2018). The significant increase in the immune parameters may be due to the cinnamaldehyde's importance of inhibition of pro-inflammatory cytokine released from mast cells in amelioration of inflammation (Deo *et al.*, 2010). Dietary rabbit's GAR plus JUN significantly increased gamma globulins by 40.3%, respectively compared to the control group (Table 5). This result probably due to the ability of JUN in treating inflammatory diseases (Jegal *et al.*, 2018). On the contrary, Toghiani *et al.* (2011) indicated that GAR additives had no significant effect on serum protein and albumin concentrations. A high level of globulin in the blood is evidence of a positive immune efficacy of an organism. The results agree with Giannenas *et al.* (2011). Since the liver performs an important function in protein metabolism, any loss of its cells is reflected in total serum proteins (Mbuh and Mbwaye, 2005).

Table (5): Serum proteins profile of rabbits supplemented with different essential oils mixture.

Item	Experimental diets				±SE	Sig.
	R1 (Control)	GAR R2	oil 0.25mg/kg diet R3	R4		
Total proteins(g/dl):	6.55 ^b	7.21 ^a	6.71 ^b	7.00 ^{ab}	0.15	*
Albumin (g/dl)	3.95	3.59	3.94	3.90	0.14	NS
Globulin (g/dl)	2.60 ^c	3.62 ^a	2.77 ^c	3.10 ^b	0.14	*
A/G ratio	1.52 ^a	1.00 ^c	1.43 ^{ab}	1.28 ^b	0.13	*
Globulin:						
Alpha1 (g/dl)	0.32 ^c	0.44 ^a	0.37 ^{bc}	0.41 ^{ab}	0.01	*
Alpha2 (g/dl)	0.81 ^{ab}	0.94 ^a	0.78 ^b	0.78 ^b	0.05	*
Beta (g/dl)	0.80 ^b	1.04 ^a	0.75 ^b	0.80 ^b	0.04	*
Gamma (g/dl)	0.67 ^c	1.21 ^a	0.94 ^b	1.11 ^{ab}	0.05	*

R1: Control diet. R2: Control diet + 0.25mg garlic oil +0.25 mg cinnamon oil. R3: Control diet + 0.25 mg garlic oil+ 0.25mg juniper oil.

R4: Control diet + 0.25mg garlic oil + 0.125mg cinnamon oil plus 0.125mg juniper oil/ kg diet.

^{a,b and c.}: means in the same row within each treatment having different superscripts differ significantly at P<0.05.

SE: Standard error of the mean. NS: Non-significant. *: P<0.05.

Effect of using of mixtures of EOs in the diets on triglycerides, renal and liver functions of rabbits:

The present results of blood parameters illustrated in Table (6) found that all groups mixture feed additives (EO) rabbit rations at R2, R3 and R4 significantly (P<0.05) lowered triglycerides, low-density lipoprotein (LDL), total cholesterol, creatinine and GOT contents, however, it significantly improved high density lipoprotein (HDL) contents compared to control ration. Dietary rabbit's GAR plus the mixture of CIN+JUN significantly decreased the triglycerides by 16.4%, total cholesterol by 44.35%, LDL by 20.18%, followed by dietary rabbit's GAR plus JUN by 13.10, 20.24 and 54.1% as well as by dietary rabbit's GAR plus CIN by 6.13, 21.67 and by 12.30%, respectively compared to the control group (Table 6).

These results in GAR groups may be due to the presence of sulfur-containing bioactive compounds in its homogenates as in layer chicken (Chowdhury *et al.*, 2002), also, allicin in GAR blocks the work of hydroxymethyl glutaryl- CoA reduction, who is the extreme significant enzyme that shares in the structure of lipids and cholesterol (Lydia, 2001) and may be due to the effect of CIN phenolic compounds for decreasing lipogenesis, increasing lipolysis, stimulating fatty acids β-oxidation and inhibiting adipocyte differentiation (Rodriguez *et al.*, 2017). Also, juniper phenolic compounds have the potential in

hypoglycemic and hypolipidemic agents (Ju *et al.*, 2008). Sher *et al.*(2012) found that rabbits fed the diet with garlic extract led to significantly ($P<0.05$) reduced triglycerides and total cholesterol.

Dietary rabbit's GAR plus CIN (R2), plus JUN (R3) plus the mixture of CIN + JUN (R4) significantly improved the kidney function by decreasing the blood urea by 35.22, 25.56 and 28.54% as well as significantly ($P<0.05$), decreased the creatinine by 11.5, 14.15 and 6.19%, matched with the liver function by decreasing the GOT by 10.62, 6.31 and 8.16%, respectively compared to the control group (Table 6). These results may be due to the administration of CIN effectively protected against the loss of these antioxidant activities, and it is well known to serve diverse biological functions, including protection of cells from oxidative damage and free radicals (Nakamura *et al.*, 2001 and Gabele *et al.*, 2009). Also, these results probably due to the JUN ameliorates gentamicin nephrotoxicity via antioxidant activity, an increase of renal glutathione content, an increase of renal antioxidant enzyme activity in male mice (Al-Attar *et al.*, 2017). Alagawany *et al.* (2016) with rabbits also reported a lowest in the SGOT levels on garlic additives in the ration.

Table (6): Blood serum constituents of rabbits supplemented with different essential oils mixture.

Item	Experimental diets				±SE	Sig.
	R1 (Control)	GAR oil 0.25mg/kg diet				
		R2	R3	R4		
Triglycerides (mg/ dl)	84.16 ^a	79.00 ^b	73.13 ^b	70.30 ^b	4.45	*
Total cholesterol (mg/ dl)	139.17 ^a	109.00 ^b	111.00 ^b	77.44 ^c	3.68	*
HDL-Cholesterol (mg/ dl)	19.40 ^d	35.46 ^a	29.56 ^b	25.32 ^c	0.74	*
LDL-Cholesterol (mg/ dl)	88.85 ^a	77.92 ^b	40.79 ^d	70.92 ^c	2.57	*
Kidney function:						
Urea (mg/dl)	53.63 ^a	34.74 ^b	39.92 ^b	38.32 ^b	2.62	*
Creatinine (mg/dl)	1.13 ^a	1.00 ^b	0.97 ^c	1.06 ^a	0.08	*
Liver function:						
GOT (U/ml)	84.87 ^a	75.85 ^b	79.51 ^b	77.94 ^b	3.62	*

R1: Control diet. R2: Control diet + 0.25mg garlic oil +0.25 mg cinnamon oil. R3: Control diet + 0.25 mg garlic oil+ 0.25mg juniper oil.

R4: Control diet + 0.25mg garlic oil + 0.125mg cinnamon oil plus 0.125mg juniper oil/ kg diet.

^{a,b,c and d}: means in the same row within each treatment having different superscripts differ significantly at $P<0.05$.

SE: Standard error of the mean. *: $P<0.05$.

Effect of using mixtures of essential oils in feed on carcass characteristics of rabbits:

Data presented in Table (7) showed that dietary treatment had a significant effect ($P<0.05$) increased on carcass weight, with or without edible offals. Dietary rabbit's GAR plus mixture CIN + JUN significantly increased the carcass weight with or without edible offals by 11.9 or by 12.6%, respectively compared to the control group. These results are expected that carcass weight is mainly associated with pre-slaughter weight but carcass yield is mainly correlated to body composition among many other factors as EOs exerts a considerable impact on the total lipids percentage (Table 6). Similar results showed that dietary inclusion of some EOs affected fat metabolism in broiler chicken (Case *et al.*, 1995). Contradictory results in the carcass characteristics of animals obtained by previous authors may be also due to different doses of essential oils, the number of experimental animals, animal age, duration of the trial period, etc., Which may be attributed to the amount of bioactive ingredients on found in garlic oil. Alcicek *et al.* (2003) recorded that herb extract resulted in improvement of the chicken carcass but had no effect on abdominal fat percentage, at the same time, add EOs at a concentration of 48 or 72 mg of an EOs combination/ kg of feed significantly improved carcass yield. The carcass yield was not significantly impacted by the addition of dietary rosemary extract (Cardinali *et al.*, 2012). In this context, Bento *et al.* (2013) found no harmful effect of EOs on carcass yield in dressing ratios and sensory quality of meat in broilers. These results were in partial harmony with Gbenga *et al.* (2009) who found that the GAR extract supplementation did not significantly affect the properties of carcass and other organs. In the same direction, Raeesi *et al.* (2010) showed that 1 or 3% GAR had no significant impact on the proportional weights of the carcass or gastrointestinal tract between groups. On the other hand, the addition of essential oil in the feed improved ($P<0.05$) the carcass yield of the broiler compared to the other groups, while it significantly decreased the gut weight of the broilers. Our results are harmonic with those of Jamroz and Kamel (2002) who showed a biggest slaughter rate in broiler chickens. In contrast to our

results on carcass yield, Mandal *et al.* (2000) showed that the carcass characteristics were not influenced by the dietary EOs treatments.

Also, data presented in Table (7) showed that dietary treatment had no significant ($P < 0.05$) effect on DP1, DP2, total edible offals includes (liver, heart, kidneys and spleen), front part, middle part and behind part. These results were in harmony with those reported by Bampidis *et al.*(2005) who found no effect of GAR diet on the characteristics of commercial sheep carcass and cuts. Sources of sulphur compounds are present in GAR and may play important roles in reducing the flavor quality of food products due to the distinctive strong smell (Yu *et al.*, 1993). In a recent study, Chaves *et al.* (2008 and 2011) also found that the addition of cinnamaldehyde and carvacrol and cinnamaldehyde doses in another experiment in a concentrate diet feeding on growing lambs did not change the flavor or taste of the meat and recorded no difference in carcass characteristics.

Table (7): Carcass characteristics of rabbits supplemented with different essential oil mixture.

Item	Experimental diets				±SE	Sig.
	R1 (Control)	GAR oil 0.25mg/kg diet				
		R2	R3	R4		
Slaughter weight (SW), g	2235.33 ^{ab}	2024.00 ^b	2087.00 ^{ab}	2486.33 ^a	120.0	*
Carcass weight (CW) g ¹	1383.33 ^b	1238.00 ^c	1250.00 ^c	1548.35 ^a	87.36	*
Carcass weight (CW) g ²	1286.00 ^b	1153.66 ^c	1162.00 ^c	1447.68 ^a	82.94	*
Dressing percentage (DP)%						
D P1, %	61.87	61.00	59.65	62.30	1.25	NS
D P2, %	57.51	56.83	55.44	58.62	1.35	NS
Front part, %	30.59	34.11	34.33	32.53	1.50	NS
Middle part, %	21.69 ^a	16.51 ^b	19.33 ^{ab}	21.20 ^a	1.09	*
Behind part, %	32.85 ^b	33.86 ^{ab}	34.75 ^{ab}	35.45 ^a	0.71	NS
Heart, %	0.59	0.57	0.44	0.47	0.05	NS
Liver, %	4.94	4.62	5.02	4.75	0.44	NS
Spleen, %	0.15	0.16	0.16	0.13	0.02	NS
Kidneys, %	1.39	1.30	1.42	1.14	0.11	NS
Total edible offal's, %	7.06	6.61	7.06	6.49	0.53	NS

R1: Control diet. R2: Control diet + 0.25mg garlic oil +0.25 mg cinnamon oil. R3: Control diet + 0.25 mg garlic oil+ 0.25mg juniper oil.

R4: Control diet + 0.25mg garlic oil + 0.125mg cinnamon oil plus 0.125mg juniper oil/ kg diet.

^{a,b and c} means in the same row within each treatment having different superscripts differ significantly at $P < 0.05$.

SE: Standard error of the mean. NS: Non-significant. *: $P < 0.05$.

CONCLUSION

From the present results, it can be concluded that the mixture of oils (garlic, cinnamon and juniper) revealed the best results and may be considered a potential growth promoter, without any negative effects on growth, carcass characteristics and blood plasma components. Therefore, medicinal and aromatic oils can be used as growth stimulants to effectively optimize diet utilization.

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تأثير التكامل بين الزيوت الأساسية للثوم والقرفة والعرعر على الأداء الإنتاجي وقابلية الهضم ومقاييس الدم للأرانب النيوزيلندية البيضاء النامية

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أجريت هذه الدراسة للوصول إلى إمكانية التأثير التكاملي بين مركبات الكبريت العضوي المتمثلة في زيت الثوم والمركبات الفينولية المتمثلة في زيت القرفة والعرعر وخليط من الزيوت الثلاثة على أداء الأرانب النامية بهدف دراسة التكامل بين الزيوت الثلاثة على أداء الأرانب وكذلك معاملات الهضم وعدد الكائنات الحية الدقيقة في الأعور ومقاييس الدم وخصائص الذبيحة.

استخدم في هذه الدراسة عدد 72 أرنب من الأرانب النيوزيلندية البيضاء قسمت إلى 4 مجموعات كل مجموعة تحتوي على 18 أرنب و كانت العلائق موزعة في جميع المجموعات كالتالي:

- 1- المجموعة الاولى غذيت على عليقة ضابطة بدون اضافات (مقارنة).
 - 2- المجموعة الثانية غذيت على العليقة المقارنة مضاف إليها 0.25 مللي جرام من زيت الثوم + 0.25 مللي جرام من زيت القرفة لكل كجم علف.
 - 3- المجموعة الثالثة غذيت على العليقة المقارنة مضاف إليها 0.25 مللي جرام من زيت الثوم + 0.25 مللي جرام من زيت العرعر لكل كجم علف.
 - 4- المجموعة الرابعة غذيت على العليقة المقارنة مضاف إليها 0.25 مللي جرام من زيت الثوم + 0.125 مللي جرام من زيت القرفة + 0.125 مللي جرام من زيت العرعر لكل كجم علف.
- وكانت اهم النتائج كالتالي:

- أدت إضافة كلا من زيت الثوم الى زيت القرفة والعرعر في المجموعة الرابعة الى زيادة عدد البكتيريا الكلية بنسبة 44.4 % والبكتيريا المحللة للسليولوز بنسبة 70% ، على التوالي بالمقارنة بالمجموعة الضابطة.
- أدى إضافة زيت الثوم في العليقة الثانية والثالثة الى تحسن كبير في هضم المادة الجافة بنسبة 41.7 و 29.1 % بالإضافة إلى زيادة قابلية هضم الدهون بنسبة 18.7 و 13.9 % على التوالي مقارنة بالمجموعة الضابطة.
- أدت معاملة الخليط في المجموعة الرابعة إلى زيادة معنوية في وزن الجسم النهائي بنسبة 11.9% ، وزيادة الوزن الكلي للجسم بنسبة 7.5% ، ومتوسط الزيادة اليومية في وزن الجسم بنسبة 51.5% ، وكذلك تحسين معدل التحويل الغذائي بنسبة 17.2% ، على التوالي مقارنة بالمجموعة الضابطة.
- خفضت المعاملة الرابعة بشكل ملحوظ من الدهون الثلاثية بنسبة 54.6% ، والكوليسترول الكلي بنسبة 67.5% ، والكوليسترول المنخفض بنسبة 52.5% تليها المعاملة الثالثة المحتوية على خليط من زيت الثوم وزيت العرعر بنسبة 53% و 32.4 و 79.2% بينما كانت نسب الانخفاض في المعاملة الثانية المحتوية على خليط من زيت الثوم وزيت القرفة 49.7، 33.2 و 30% على التوالي مقارنة بالمجموعة الضابطة.
- أدت المعاملة الرابعة للأرانب المغذاة على خليط من الزيوت الثلاثة إلى زيادة معنوية في وزن الذبيحة مع او بدون الاحشاء الداخلية المأكولة بنسبة 11.9% أو 12.6% على التوالي مقارنة بمجموعة المقارنة.

* من خلال هذه النتائج وجد ان المعاملة الرابعة التي كانت تحتوي على خليط من الزيوت الثلاثة أدت الى تحسن كبير وزيادة في عدد الكائنات الحية الدقيقة في الأعور وزيادة معدلات النمو وكذلك تحسين معاملات الهضم وبعض خصائص الذبيحة وكذلك تأثيرها على مقاييس الدم وذلك بتقليل نسبة الدهون الكلية والكوليسترول في الدم.