

## **EFFECT OF ADDING CINNAMON, GARLIC AND JUNIPER ESSENTIAL OILS ON PRODUCTIVE PERFORMANCE OF NEW-ZEALAND WHITE RABBITS**

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### **SUMMARY**

A total number of 72 male New-Zealand White (NZW) rabbits after weaning were used to study the effect of cinnamon, garlic and juniper essential oils (EOs) as feed additives at level of 0.5 ml/kg diet on rabbit's nutrients digestibility, growth performance, caecum microorganism's count, blood serum constituents and carcass characteristics. Rabbits were classified into 4 equal groups divided into 3 replicates, 6 rabbits each, till age 8 weeks. The 1<sup>st</sup> group received the basal diet (G1). The other three groups (G2<sup>nd</sup>-G4<sup>th</sup>) received the basal diet with cinnamon, garlic and juniper (EOs) at 0.5ml/kg diet v/w, respectively. Juniper EO significantly decreased the CP digestibility by 5.5% and the CF digestibility by 3.3% compared to the control group. Garlic EO significantly increased the total body weight by 12.4% and the average body weight gain by 12.4%, compared to the control group. Adding cinnamon and juniper EOs to diets led to a significant decrease in the total daily dry matter intake compared to the control group. The results also indicated that adding cinnamon, garlic and juniper EOs significantly increased the total bacterial count by 22.2, 70 and 33.3% and the cellulolytic bacteria by 50, 100 and 60%, respectively compared to the control group. Juniper oil significantly increased the levels of serum albumin and improved feed conversion by 29.4% compared to the control group. Cinnamon and juniper oils significantly decreased the serum levels of triglycerides, total cholesterol, GOT and urea, while significantly increase HDL-cholesterol compared to the control group.

**Keywords:** *New-Zealand White (NZW) rabbits essential oils, digestibility, growth performance, serum biochemical parameters and carcass characteristics.*

### **INTRODUCTION**

Recently, it recommended caution in their use, and pay attention to the potential risks of using antibiotics as growth promoters the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have indicated this (FAO/WHO, 2004). With the ban on the use of use antibiotics by the European Commission, the livestock scientists have shifted their attention towards herbal feed additives/herbal growth promoters (Jayasena and Jo, 2013). Pay nutrition experts and feed manufacturers to develop and produce other alternatives such as organic acids, probiotics or prebiotics and feed enzymes as a result of the restrictions imposed on the use of antibiotics as additives for feed. These materials are firm in the nutrition of all animals. In contrast, plant extracts, and especially EOs, are a new category of feed additives and awareness regarding their modes of action and application aspects are still somewhat rudimentary (Windisch *et al.*, 2008). In recent years, EOs has attracted increased attention from the poultry industries (Başer and Demirci, 2007), which are concentrated hydrophobic liquids containing aromatic volatile compounds obtained from medicinal plants (Mathe, 2009).

Numerous studies include documented the utilize of plant feed additives (essential oils) as a growth promoter (Kim *et al.*, 2013; Elagib *et al.*, 2013 and Lee *et al.*, 2016). Herbal growth promoters serve as

appetizers, digestive stimulants, antibacterial, antiviral, anthelmintic, anti-inflammatory and also possess immuno-stimulation properties (Platel *et al.*, 2002 and Wenk, 2003). The herbal EOs compounds can penetrate the plasma membrane of pathogenic bacteria because of their lipophilic characteristic (Nogueira, 2010) this disrupts the structure of the polysaccharide, fatty acid and phosphorous layers, making the membrane more permeable (Bakkali *et al.*, 2008). These EOs of cinnamon (Borges *et al.*, 2016), garlic (Rees *et al.*, 1993) and juniper (Stassi *et al.*, 1996) have antimicrobial potential, thus herbal medication in the treatment of several diseases are on the rise.

Cinnamon (*cinnamomum*) the eternal plant of tropical medicines belongs to the *Lauraceae* family (Rao and Gan 2014). It primarily consists of essential oils and other constituents contain large quantities of terpenes and aromatic compounds CPC (2010). The most abundant component of the bark oil was trans-cinnamaldehyde 74.2% (Yan-qun *et al.*, 2013) which was proven to be active against microorganisms (Chen *et al.*, 2015) and eugenol 7% (Sousa *et al.*, 2004) that has strong antimicrobial activity against gram-positive and gram-negative bacteria (Almariri and Safi, 2014) as well as anti-parasitic (Jakhatia *et al.*, 2010). Besides, cinnamon has antioxidant, anti-inflammatory, antimicrobial and lipid-lowering properties, so it can use to treat certain inflammatory conditions (Mahmoudv *et al.*, 2017).

Garlic (*Allium sativum*) belongs to the *Amaryllidaceae* family and has been a subject of considerable interest as a medicinal and therapeutic agent globally since ancient times. The main pharmacological effects of garlic are attributed to 'allicin', an organosulphur compound that exhibits antibacterial (Lanzotti, 2006 and Toghyani *et al.*, 2011), antifungal, anti-parasitic, antiviral (Ankri and Mirelman, 1999), antioxidant (Banerjee *et al.*, 2003 and Lee *et al.*, 2009), hypocholesterolemic (Gupta and Porter, 2001). Garlic can increase the total protein, albumin and globulin concentrations as reported by Hassan and Abdel-Raheem (2013).

Juniper (*Juniperus communis*) belongs to the *Cupressaceae* family; has antimicrobial, anti-rheumatic, analgesic (Asili *et al.*, 2008). It contains approximately 70 species (Khan *et al.*, 2012). The hydrophobic properties of CIN, GAR and JUN EOs interact with the pathogenic bacteria cell membrane causes disruption and inhibition of energy generation (Gill and Holley, 2004) causing the death of cells (Paul *et al.*, 2011) and consequently and allow the improving digestion and increased immunity by beneficial bacteria (Borges *et al.*, 2016).

This study aims to evaluate the effects of CIN, GAR and JUN (EOs) as feed additives on the nutrients digestibility, growth performance, microbial environment in rabbits and to assess blood biochemical parameters and carcass properties.

## **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 EOs of CIN, GAR and JUN in growing rabbit diets using seventy-two male NZW rabbits after weaning with an average body weight of  $817.58 \pm 37.61$ g. Rabbits were housed in individual wire cages and divided into four equal treatment groups of 18 rabbits each three replicates of six each.

### ***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.

### ***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). The feeding period was extended for 45 days, and the experimental groups were classified as follows: The 1<sup>st</sup> group received the basal diet (G1). The other three groups (G2<sup>nd</sup>–G4<sup>th</sup>) received the basal diet with CIN, GAR and JUN essential oils at 0.5

ml/kg diet v/w, respectively. The CIN, GAR and JUN EOs used in this study were sprayed by 0.5ml/kg diet 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. 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.

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

Ingredient	Content (%)
Clover hay	32.00
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
Nitrogen free extract (NFE)	57.39
Ash	9.11
Growth energy (Kcal/kg DM)**	4176.93
Digestible energy (Kcal/kg DM)***	2481.12

\* 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 %).

#### **Digestibility trial:**

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 offal's 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 were 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 the method of Hoskins (1934). The 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 were 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 protein was performed according to the method of Henry *et al.* (1974), 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's 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,  $\mu$ =overall mean,  $T_i$ =effect of experimental rations for  $i = 1-4$ .  $e_{ij}$  = the experimental error.

## **RESULTS AND DISCUSSION**

#### ***Effect of essential oil supplementation on rabbit's digestibility:***

The digestibility of nutrient components of different diets is presented in Table (2). The results indicated that the addition of cinnamon, garlic and juniper essential oils did not affect the digestibility of DM, OM, EE and NFE. While, EOs supplementation affected the digestibility of CP and CF especially with G3 diet due to increased amounts of protein available at the cellular level for deposition in the body tissues. These results were supported by the findings of CF digestibility agrees with Patra *et al.* (2001); Shehata *et al.* (2003) and Hernandez *et al.* (2004) who indicated that the great improvement in CP and CF digestibility resulted from adding garlic at different levels. The results also, showed an improvement in the complete digestion of nutrients, especially CP, as a result of consuming garlic extract. The herbal effects of garlic powder improve digestibility due to the increase in the number of microbes, especially the number of bacteria such as *E. coli*, *Clostridium spp.* and *Enterococci*. Many authors have suggested that dietary essential oils improve the performance of birds, because these substances stimulate the secretion of internal digestive enzymes, which increases the digestion of nutrients or the rate of gut passage or feed intake (Muhl and Liebert, 2007).

Juniper EOs significantly ( $P < 0.05$ ) decreased the CP digestibility by 5.5% and the CF digestibility by 3.3%, compared to the control group (Table 2). These results probably because juniper act to protect the gastric mucosa from the conclusive effect of hydrochloric acid and the digestive action of proteolysis enzymes, as a result of the juniper physiological secretion from goblet and mucous cells (Ben Ali *et al.*, 2015), which also may attribute to the significant decreasing value by 5.5% of DCP compared to the control group. In other words, may be due to that juniper EO possesses anti-glycation properties (Asgary *et al.*, 2014). The nutritive values of the different experimental diets are shown in Table (2). The results stated that addition of cinnamon, garlic and juniper EOs did not effect on the total digestible of nutrients (TDN). While, the addition of juniper EO (G4) significant ( $P < 0.05$ ) decreased DCP. This result agrees with the result of Patra *et al.* (2001) and Shehata *et al.* (2003) who reported that the addition of garlic improved DCP and TDN significantly ( $P < 0.05$ ). These results might be due to the retrieval of important

protein which increases glutathione enzymes in the liver which safeguard the cells from oxidative damage and play a vital role in detoxification, better organs function and immunity and inhibits lipid peroxidation.

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

Item	Experimental diets				±SE	Sig.
	G1	G2	G3	G4		
Body weight, kg	2.81	2.32	2.09	2.37	0.09	NS
Dry matter intake, g/h/d	143.24 <sup>a</sup>	115.19 <sup>b</sup>	127.70 <sup>ab</sup>	92.60 <sup>c</sup>	6.21	*
Dry matter, g/ kg body weight	61.68 <sup>a</sup>	55.22 <sup>b</sup>	54.27 <sup>b</sup>	49.70 <sup>c</sup>	1.75	*
Nutrients digestibility,%:						
DM	60.22	60.23	60.85	55.97	0.98	NS
OM	66.52	65.64	67.15	62.10	0.92	NS
CP	78.37 <sup>a</sup>	77.99 <sup>a</sup>	79.23 <sup>a</sup>	74.08 <sup>b</sup>	0.69	*
CF	33.08 <sup>b</sup>	31.75 <sup>b</sup>	37.24 <sup>a</sup>	31.97 <sup>b</sup>	1.86	*
EE	68.72	77.32	78.56	75.63	1.54	NS
NFE	74.03	72.42	72.67	67.59	1.07	NS
Feeding value,%:						
TDN	57.17	56.82	58.11	53.86	0.76	NS
DCP	12.31 <sup>a</sup>	12.24 <sup>a</sup>	12.44 <sup>a</sup>	11.63 <sup>b</sup>	0.11	*

G1: Control diet. G2: Control diet + 0.5ml cinnamon oil. G3: Control diet + 0.5ml garlic oil .G4: Control diet + 0.5ml juniper oil/kg diet.

<sup>a, b and c</sup>: 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 essential oil supplementation on rabbit's growth performance:**

The effect of different supplementation (G2, G3 and G4) of EOs on the growth performance of rabbits is shown in Table (3). The average final live weights of rabbits for different groups were 2122.33, 2126.17, 2268.17 and 2164.83g for G1, G2, G3 and G4, respectively and the results did not significantly differ ( $P > 0.05$ ) between the different groups. The average live body weight of the rabbits was 29.14, 29.25, 32.74 and 29.13 g/h/d for G1, G2, G3 and G4, respectively (Table 3). Garlic EO significantly ( $P < 0.05$ ) increased the total body weight by 12.4% and the average body weight gain by 12.4%, compared to the control group. This improvement in LBW with phytochemical additives supplementation may also be due to the provision of some compounds that increase the digestion and absorption of certain nutrients in the diets, which may be attributed to the bioactive ingredients (curcuminoids and allicin) present in garlic that cause greater efficiency and also in enhanced growth. These results partially agree with Gbenga *et al.* (2009) who indicated that BWG, FI and FCR were not statistically influenced by dietary garlic supplementation; they were 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. Adibmoradi *et al.* (2006) found that garlic supplements a positive effect on the histological structure of the digestive system occurs, which may increase digestion and nutrient absorption, and improve product performance when you add them up (from 5 to 20 g / kg). Also, may be due to the series of its biological benefits as antioxidant potential (Asdaq, 2015), antimicrobial activity (Ponmurugan and Shyamkumar 2012). Juniper EOs significantly ( $P < 0.05$ ) improved feed conversion compared to the other groups (Table 3). This result may be due to its effect in the treatment of intestinal worms, heal wounds cure liver diseases (Loizzo *et al.*, 2007) and abdominal spasm (Khan *et al.*, 2012). The useful impact of plant feed additives on enhanced performance and FCR can also be demonstrated by the antioxidant efficiency of the bioactive compounds like cineol, thymol, pinene and carvacrol (Faleiro *et al.*, 2005). As well as by improving the activity of the enzyme in the gut, beneficial stimulation and inhibition of pathogenic bacteria eventually resulting in improved absorption and use of nutrients (Windisch *et al.*, 2008 and Frankic *et al.*, 2009).

#### **Effect of essential oil supplementation on rabbits dry matter intake (DMI) and feed conversion ratio (FCR):**

All treatments, except garlic, significantly decreased ( $P < 0.05$ ) DMI compared to the control group (Table 3). The daily DMI was 105.0, 90.0, 96.49 and 73.63 g/h/d for rabbits fed diets G1, G2, G3 and G4, respectively among the treatment groups. Juniper EO significantly ( $P < 0.05$ ) improved the FCR by 29.4% compared to the control group. On the other hand, DMI was significantly affected by the essential oils

added in the treatment compared, except garlic oil to the control group. Feed intake was significantly reduced when fed was supplemented with caraway oil (100 mg/kg) or fennel (100 mg/kg) suggesting that palatability was low due to the flavour in feed experiment conducted in developing pigs (Schone *et al.*, 2006). Essential oils in addition to the rabbit diet at 0.05 g/ kg diet had no significant effect on feed consumption, except for feed consumption for the control group. In this regard, Denli *et al.* (2004) reported that adding fennel EO to a quail diet improves the FCR. Also, the addition of oregano or thyme and their essential oils decreased the daily feed intake and significantly enhanced the feed conversion ratio in chicken (Halle *et al.* 2004).

Herbal feed additive improved ADG, FCR and increased liveability for rabbits (El-Kholy *et al.*, 2012 and Zeweil *et al.*, 2013 and 2016a and b). It is noteworthy that many difficulties face the supply of herbs and dietary essential oils to animals, because of tastes and odours emanated by the active substances contained in the plants, which inhibit intake by the animal. In other words, some herbs can be scarcely appetizing. In fact, before approaching the feed, the animals are strongly influenced by the aroma that it emanates from.

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

Item	Experimental diets				±SE	Sig.
	G1	G2	G3	G4		
Initial body weight, g	811.17	810.00	795.00	854.17	37.61	NS
Final body weight, g	2122.33	2126.17	2268.17	2164.83	37.17	NS
Total body weight gain, g	1311.16 <sup>b</sup>	1316.17 <sup>b</sup>	1473.17 <sup>a</sup>	1310.66 <sup>b</sup>	25.59	*
Average body weight gain, g	29.14 <sup>b</sup>	29.25 <sup>b</sup>	32.74 <sup>a</sup>	29.13 <sup>b</sup>	0.57	*
Dry matter intake, g/h/d	105.00 <sup>a</sup>	90.00 <sup>b</sup>	96.49 <sup>ab</sup>	73.63 <sup>c</sup>	2.98	*
Dry matter intake, g/kg body weight	72.74 <sup>a</sup>	62.40 <sup>a</sup>	63.86 <sup>a</sup>	49.06 <sup>b</sup>	2.67	*
Feed conversion, kg DMI/ kg gain	3.60 <sup>a</sup>	3.07 <sup>b</sup>	2.97 <sup>b</sup>	2.54 <sup>c</sup>	0.09	*

G1: Control diet. G2: Control diet + 0.5ml cinnamon oil. G3: Control diet + 0.5ml garlic oil .G4: Control diet + 0.5ml juniper oil/kg diet .

<sup>a,b and c</sup>: 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 essential oil supplementation on rabbit's microorganism's count:**

The results of the current study revealed a significant ( $P < 0.05$ ) increased in the count of total bacterial and the cellulolytic bacteria, respectively, in garlic, juniper and cinnamon EOs supplemented rabbits compared with the control group (Table 4). Increasing the availability of nutrients for animal use leads to a positive decrease in pathogenic bacteria, thus reducing food competition and preventing many intestinal diseases (Yitbarek, 2015).

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

Item	Experimental diets				±SE	Sig.
	G1	G2	G3	G4		
Total bacterial count, 10 <sup>5</sup>	45.00 <sup>d</sup>	55.00 <sup>c</sup>	76.67 <sup>a</sup>	60.00 <sup>b</sup>	3.56	*
Cellulolytic bacteria, 10 <sup>5</sup>	50.00 <sup>c</sup>	75.00 <sup>b</sup>	100.00 <sup>a</sup>	80.00 <sup>b</sup>	0.34	*

G1: Control diet. G2: Control diet + 0.5ml cinnamon oil. G3: Control diet + 0.5ml garlic oil .G4: Control diet + 0.5ml juniper oil/kg diet .

<sup>a,b,c and d</sup> means in the same row within each treatment having different superscripts differ significantly at  $P < 0.05$ . SE: Standard error of the mean. \*  $P < 0.05$ .

In garlic EO group which showing significantly increase, these results may be due to the effect of garlic oil in disruptions of the normal cellular metabolism and physical functions of the pathogenic bacteria (Gow, 2017) as well as the membranes of organelles such as the mitochondria resulting in destruction and ultimately cell death (Li *et al.*, 2016). In other words, garlic oil caused down- regulation of pathogenicity-related genes and could reduce the virulence and pathogenicity of pathogenic bacteria (Li *et al.*, 2016) via raises of methyl sulphides which has a high relative antimicrobial activity (O'Gara *et al.*, 2000). In juniper

EO (G4) significantly increased, may be due to the hydrophobic nature (Ramdani *et al.*, 2013) and to the phenolic contents in juniper (Liu *et al.*, 2017), suggesting that antibacterial activity (Stassi *et al.*, 1996). In cinnamon EO (G2) may be due to the cinnamaldehyde transformation to a new product which is responsible for the antibacterial activity in the cecum and inhibit selected microbes and consequently may improve growth performance (Yang *et al.*, 2010 and Stella *et al.*, 2017).

**Effect of essential oil supplementation on rabbit's blood serum parameters:**

The results are showing a significant ( $P<0.05$ ) increased in the serum albumin value noticed in the juniper essential oil group compared to the control group (Table 5). The results of protein electrophoresis showed a significant decrease in alpha globulin in G2 compared with the control group (Table 5). This result may be due to its ability to protein synthesis that has been advanced as a contributory hepatoprotective mechanism that accelerates the regeneration process and the production of liver cells (Hatano *et al.*, 1989) or may be due to its effect in the treatment of intestinal worms (Loizzo *et al.*, 2007). The health condition is usually related to the biochemical parameters of the blood. Animals' physiological, pathological and nutritional status is good indicators of them and can use them to demonstrate the influence of nutritional factors and additives available in the diet. Adding garlic to the main diet showed a significant positive effect on blood measurements which is in accordance by Konjufca *et al.* (1997). Total protein measurements can reflect protein synthesis, nutritional status, dehydration, kidney disease, liver disease and many other conditions (Acar *et al.*, 2018). The addition of cinnamon EO to rabbit diets ( $P>0.05$ ) increased plasma globulin values in conjunction with no significant effect on plasma albumin and that indicated improve immune response due to the protective and the potential antioxidative effect of cinnamon that leads to decrease A/G ratio (Hind *et al.*,2012; Tollba *et al.*,2010 and Hussein, 2010).

Increase plasma globulin indicated improving immune response in rabbits (Acar *et al.*, 2018). In this respect, garlic supplements increase plasma globulin and that may be due to organosulphur compounds in garlic and their hepatoprotective effects (Ajayi *et al.*, 2009). These findings corroborate with Alagawany *et al.* (2016). At the same time, decreased A/G ratio indicated improving immune response in the group that received garlic supplement (Venkatesh *et al.*, 2002). Regarding juniper EO, significantly increased serum albumin value due to the addition of juniper oil as the result of its hepatoprotective effect that leads to improve the regeneration process of hepatic cells (Hatano *et al.*, 1989).

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

Item	Experimental diets				±SE	Sig.
	G1	G2	G3	G4		
Total proteins (g/dl)	6.55	6.76	6.50	6.98	0.15	NS
Albumin (g/dl)	3.95 <sup>b</sup>	4.12 <sup>b</sup>	3.86 <sup>b</sup>	4.61 <sup>a</sup>	0.14	*
Globulins (g/dl)	2.60	2.64	2.65	2.37	0.14	NS
A/G ratio	1.52 <sup>ab</sup>	1.24 <sup>b</sup>	1.46 <sup>b</sup>	1.98 <sup>a</sup>	0.13	*
Globulins (g/dl)						
Alpha1 (g/dl)	0.32 <sup>a</sup>	0.25 <sup>b</sup>	0.33 <sup>a</sup>	0.33 <sup>a</sup>	0.01	**
Alpha2 (g/dl)	0.81	0.79	0.78	0.81	0.05	NS
Beta (g/dl)	0.80	0.73	0.73	0.66	0.04	NS
Gamma (g/dl)	0.67 <sup>bc</sup>	0.87 <sup>a</sup>	0.81 <sup>ab</sup>	0.57 <sup>c</sup>	0.05	*

G1: Control diet. G2: Control diet + 0.5ml cinnamon oil. G3: Control diet + 0.5ml garlic oil .G4: Control diet + 0.5ml 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. \*: $P<0.05$ . \*\*: $P<0.01$ .

**Effect of essential oil supplementation on rabbit's blood serum lipids, kidney and liver function:**

Results of lipid profile, kidney functions and liver enzyme showed a significant ( $P<0.05$ )decreased in serum total cholesterol (TC), triglycerides (TG), LDL and GOT in all groups, while HDL showed a significant increased in all groups compared to the control group (Table 6). The significant decreased of alkaline phosphatase (ALP) value may be due to its effect on the hepatocytes functions (Naik and Panda 2008). Garlic EOs significantly decreased the LDL- cholesterol by 63.11% and the GOT by 24.12% as well as significantly increased the HDL- cholesterol by 30.52%, respectively compared to the control group (Table 6).

The HDL-cholesterol levels were significantly ( $P<0.05$ ) increased in the garlic supplemented rabbits, as compared with the non-supplemented rabbits (Table 6). This increase might be due to the

hypocholesterolemic mechanism and the hypolipidemic action of garlic. ‘Allicin’ present in garlic combines with the –SH (sulphadryl) group that is important in the activation of acetyl CoA which is essential for the biosynthesis of cholesterol (Puvaca *et al.*, 2014).

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

Item	Experimental diets				±SE	Sig.
	G1	G2	G3	G4		
Triglycerides (mg/ dl)	84.16 <sup>a</sup>	80.72 <sup>b</sup>	83.16 <sup>a</sup>	68.67 <sup>c</sup>	2.33	*
Total cholesterol (mg/ dl)	139.17 <sup>a</sup>	77.44 <sup>c</sup>	79.91 <sup>c</sup>	121.87 <sup>b</sup>	3.5	*
HDL-Cholesterol (mg/ dl)	19.40 <sup>b</sup>	25.15 <sup>a</sup>	25.32 <sup>a</sup>	25.32 <sup>a</sup>	1.65	*
LDL-Cholesterol (mg/ dl)	88.85 <sup>a</sup>	38.51 <sup>b</sup>	32.78 <sup>c</sup>	85.32 <sup>a</sup>	2.54	*
Kidney function						
Urea (mg/dl)	53.63 <sup>a</sup>	38.78 <sup>d</sup>	41.95 <sup>c</sup>	50.06 <sup>b</sup>	2.78	*
Creatinine (mg/dl)	1.13 <sup>b</sup>	1.05 <sup>c</sup>	1.38 <sup>a</sup>	1.31 <sup>a</sup>	0.06	*
Liver function						
GOT (U/ml)	84.87 <sup>a</sup>	67.77 <sup>c</sup>	64.35 <sup>c</sup>	76.51 <sup>b</sup>	3.15	*

G1: Control diet. G2: Control diet + 0.5ml cinnamon oil. G3: Control diet + 0.5ml garlic oil .G4: Control diet + 0.5ml juniper oil/kg diet.

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

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

Serum cholesterol rate was significantly ( $P < 0.05$ ) lowest by garlic supplementation to growing rabbit diet (Yalcin *et al.*, 2006). The reduction of serum cholesterol observed could be returned to the lowering of synthetic enzyme efficiency as suggested by Chowdhury *et al.* (2002). Alagawany *et al.* (2016) reported that garlic reduced the lipid profile in blood and enhanced the immunity responses, lipid peroxidation in the liver and increased hepatic antioxidant effectiveness in treated rabbits.

Increasing antioxidant properties by increasing glutathione levels and antioxidant enzyme activity due to the presence of organosulfur compounds in garlic oil (Lee *et al.*, 2009 and Anwar and Meki, 2003). The current results are in agreement with the findings of Mirhadi *et al.* (1991) in rabbits and Faisal *et al.* (2017) in male albino rats. Juniper EO significantly decreased triglycerides by 18.4%, total cholesterol by 12.4% and the LDL- cholesterol by 4% (un-significant) and the ALP by 9.9% as well as significantly increased the HDL- cholesterol by 30.5%, respectively compared to the control group (Table 6). Similar results showed that juniper has a greater hypolipidemic effect (Ju *et al.*, 2008). In G4 (Juniper EO) total cholesterol and triglycerides values significantly decrease compared with control and other groups, respectively. Nasri *et al.* (2011) mentioned that juniper oil is wealthy in linoleic acid (LA), oleic acid (OA) and linolenic acid (LNA), so consuming feed rich in OA, LA and LNA reduced the serum lipid level (Fernandez and West, 2005). Cinnamon EO supplementation increased serum HDL-C and decreased TC, TG and LDL-C (Khan *et al.*, 2003). It has been reported that cinnamaldehyde may stimulate lipolysis via activation of adenosine monophosphate-activated protein kinase (AMPK) which is involved in the maintenance of lipid and cholesterol homeostasis (Shen *et al.*, 2014).

Regarding the effect of EO on the liver enzyme in G2 (cinnamon oil) decreased value of serum GOT, this indicated that improvement of lipid profile indicates the hepato-protection effect of cinnamon of liver function in lipid metabolism and it reduces cholesterol biosynthesis (Razieh *et al.*, 2019). Regarding the effect of garlic in the G3 on the activity of liver enzymes, a significant decrease of GOT was recorded which may be due to the effect of organosulphur compound present in garlic that indirectly protecting the liver from any damage maintaining its integrity and function. Extract of cinnamon can lower serum urea and creatinine values significantly the active compounds such as polyphenols from cinnamon cinnamaldehyde as an antioxidant and anti-inflammatory to improve kidney functions. The mechanism whose role is to suppress oxidative stress from a variety of oxidative reactions that occur in the kidney (Kang *et al.*, 2016). Aprioku and Amah (2017) found that garlic reduces liver decline and enhanced the biochemical plasma workers of hepatic functions, such as urea, creatinine and aspartate transaminase.

**Effect of essential oil supplementation on rabbit’s carcass characteristics:**

Cinnamon, garlic and juniper EOs significantly increased the carcass front part weight by 11.4, 27.4 and 10.7%, respectively compared to the control. Garlic and juniper oils significantly increased the liver weight by 4.7 and 6.1%, respectively compared to the control group. Cinnamon oil significantly increased the spleen weight by 6.7% compared to the control group. Carcass weight, middle part and back part (%) there were no significant differences ( $P > 0.05$ ) between supplemented groups as compared to the control.

This increase was opposed by a decrease in both heart weight as well as total edible offal's weight by compared to the control group (Table7). The findings of Dieumou *et al.* (2009) and Fadlalla *et al.* (2010), due to the garlic powder it contained, the diet of broiler chickens reported a nonsignificant effect on broiler carcass characteristics.

**Table (7): Carcass characteristics of rabbits fed diets supplemented with different essential oils.**

Item	Experimental diets				±SE	Sig.
	G1	G2	G3	G4		
Carcass weight /live body weight (%)	61.87	60.86	60.67	62.4	0.48	NS
Carcass front part /live body weight (%)	30.59 <sup>c</sup>	34.08 <sup>b</sup>	38.96 <sup>a</sup>	33.87 <sup>b</sup>	1.11	**
Carcass middle part /live body weight (%)	21.69	18.86	18.80	20.31	2.54	NS
Carcass back part /live body weight (%)	32.85	35.15	35.46	35.59	3.38	NS
Heart/ live body weight (%)	0.59 <sup>a</sup>	0.49 <sup>c</sup>	0.42 <sup>d</sup>	0.52 <sup>b</sup>	0.12	*
Liver /live body weight (%)	4.94 <sup>b</sup>	4.96 <sup>b</sup>	5.17 <sup>a</sup>	5.24 <sup>a</sup>	0.52	*
Spleen /live body weight (%)	0.15 <sup>b</sup>	0.16 <sup>a</sup>	0.13 <sup>c</sup>	0.15 <sup>b</sup>	0.00	*
Kidneys /live body weight (%)	1.39 <sup>a</sup>	1.31 <sup>b</sup>	1.16 <sup>c</sup>	1.12 <sup>c</sup>	0.03	*
Total edible offals (%)	7.06 <sup>a</sup>	6.93 <sup>b</sup>	6.89 <sup>b</sup>	7.13 <sup>a</sup>	2.34	*

G1: Control diet. G2: Control diet + 0.5ml cinnamon oil. G3: Control diet + 0.5ml garlic oil .G4: Control diet + 0.5ml juniper oil/kg diet .

<sup>a,b,c and d</sup>: 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. \*:  $P < 0.05$ . \*\*:  $P < 0.01$ .

Garlic and juniper oil significantly increased liver weight compared to the control and cinammoun groups. These results may be due to that garlic and juniper EOs can ameliorate increased serum liver and kidney activities in which because it is the largest gland found in the animal's body, and therefore the liver plays an essential role in digesting nutrients, metabolizing proteins and fats, and producing enzymes (Peng *et al.*, 2016) and these EOs may contribute in the healing of hepatic parenchyma and the regeneration of hepatocytes which may be caused by any stress conditions on the rabbit (Lesiuk *et al.*, 2003). In other words, the garlic EO can ameliorate increased serum liver and kidney activities via its ability to improve hepatocellular architecture induced elevation of serum biochemical parameters (Zhang *et al.*, 2012) and in juniper may be due to its role recover several enzymatic efficiencies and in ameliorating the toxic and critical disorders induced on the liver and kidney and a high antioxidant activity due to its component of flavonoids (Ali *et al.*, 2010). Cinnamon oil significantly increased the spleen compared to the control group. This result may be due to the cinnamaldehyde can ameliorate the toxicity of chemical toxins in body organs in particular spleen as an important vital organ of the immune system (Aswar *et al.*, 2015).

## CONCLUSION

In general, it is recommended that the use of cinnamon, garlic and juniper essential oils as feed additives at 0.5ml /kg in rabbit diets without adverse effect on the rabbit performance. However, it was found that the use of garlic oil was more effective in digestion, growth and some blood measurements and some carcass characteristics than cinnamon and juniper oil, while all oils showed their superiority over the control treatment.

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## تأثير اضافة الزيوت الأساسية لزيت القرقة والثوم والعرعر على الأداء الإنتاجي وقابلية الهضم ومقاييس الدم للأرانب النيوزيلندية البيضاء النامية

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أجريت هذه الدراسة للتعرف على تأثير إضافة الزيوت الأساسية بشكل منفرد وتأثير المركبات الفينولية المتمثلة في زيت القرقة والعرعر وكذلك تأثير المركبات الكبريتية العضوية المتمثلة في زيت الثوم على أداء الأرانب النيوزيلندية البيضاء النامية لدراسة تأثير الزيوت الثلاثة على معاملات الهضم وعدد الكائنات الحية الدقيقة في الأعور ومقاييس الدم وخصائص الذبيحة.

استخدم في هذه الدراسة عدد 72 أرنب من الأرانب النيوزيلندية البيضاء قسمت الى 4 مجموعات كل مجموعة تحتوى على 3 مكررات كل مكررة تحتوى على 6 أرانب وكانت العلائق موزعة في المجموعات كالتالى:

- 1- المجموعة الأولى غذيت على عليقة ضابطة بدون اضافة ( عليقة مقارنة).
- 2- المجموعة الثانية غذيت على العليقة المقارنة مضاف اليها 0.5 مللى جرام من زيت القرقة لكل كجم علف.
- 3- المجموعة الثالثة غذيت على العليقة المقارنة مضاف اليها 0.5 مللى جرام من زيت الثوم لكل كجم علف.
- 4- المجموعة الرابعة غذيت على العليقة المقارنة مضاف اليها 0.5 مللى جرام من زيت العرعر لكل كجم علف.

وكانت اهم النتائج كالتالى:

- إضافة زيت القرقة والثوم والعرعر أدى إلى زيادة معنوية في تعداد البكتيريا الكلية بنسبة 22.2 و 70 و 33.3% والبكتيريا المحللة للسيليلوز بنسبة 50 و 100 و 60% على التوالي مقارنة بمجموعة المقارنة.
- ادت اضافة زيت العرعر الى تقليل معاملات هضم البروتين بشكل ملحوظ بنسبة 5.5 % وهضم الالياف بنسبة 3.3 % على التوالي مقارنة بمجموعة المقارنة.
- أدت اضافة زيت الثوم إلى زيادة معنوية في وزن الجسم الكلى ومتوسط وزن الجسم مقارنة بمجموعة المقارنة.
- أدت إضافة كلا من زيت القرقة والعرعر إلى العلائق إلى انخفاض معنوي في إجمالي المادة الجافة اليومية مقارنة بمجموعة المقارنة.
- اضافة زيت العرعر الى العلائق يزيد بشكل كبير من مستويات الألبومين في الدم ويحسن كفاءة تحويل الغذاء بنسبة 29.4% مقارنة بمجموعة المقارنة.
- كلا من زيت القرقة والعرعر يقللان بشكل معنوي من مستويات الدهون الثلاثية والكوليسترول الكلى ووظائف الكبد واليوربا في الدم ، بينما أدى إلى زيادة معنوية في الكوليسترول الجيد مقارنة بمجموعة المقارنة.