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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2019 16(2): 1808-1823. **OPEN ACCESS**

Inclusion of natural bioactive mixture in drinking water: 1 Nutritional impact on Growth performance, carcass characteristics, ceacum, blood and microbiological parameters of Rex rabbits

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This work carried out to investigate the impact of adding natural bioactive mixture juice composed of lemon, onion and garlic (LOG) juice on growth performance, mortality rate, carcass characteristics, some blood constituents, ceacum parameters and microbial activity. A total number of three hundred Rex rabbits aged 4 to 5 weeks (772±22 g) were divided into four equal comparable experimental groups (75 in each). Feeding trial lasted for 8 weeks (56 days) and the first experimental group rabbits received the basal diet that formulated to cover the requirements of growing rabbits and considered as (control group), meanwhile, drinking water was offered ad libtum without containing any additives (Zero LOG). On the other hand, the 2*nd*; 3*rd* and 4*th* groups were also fed the basal diet; meanwhile drinking water was added at levels 10, 20 and 30 ml LOG/ liter of drinking water for the 2*nd*; 3*rd* and 4*th* groups, respectively. The results showed that inclusion LOG at different levels significantly (P<0.05) increased average daily gain (ADG) compared to control. This increasing reaching to 13.7, 24.6 and 22.2% for 10, 20 and 30 ml LOG, respectively in comparison with control. Daily dry matter intake (DMI) was not affected by adding LOG at different levels, the average daily DMI was varied from 94 to 98 g/head/day among the different experimental group rabbits. Feed conversion that significantly (P<0.05) improved with adding LOG at different levels and through out the 2*nd* week, 4*th* week, 6*th* week and 8*th* week compared to control. Mortality rate was decreased with increasing the level of LOG addition. Inclusion LOG at different level significantly increased both carcass weight (CW) and Dressing percentages (DP). Increasing level of LOG increased values of red blood cells, white blood cells, hemoglobin, total protein, Albumin, globulin and albumin: globulin ratio were significantly (P<0.05) increased. Meanwhile it significantly (P<0.05) decreased values of blood NH3, blood urea, GOT, GPT, total lipids, phospholipids, triglycerides, HDL-cholesterol and total cholesterol. Caecal pH insignificantly decreased with adding LOG in drinking water up to 20 ml LOG/ liter drinking water. Meanwhile, adding LOG at different levels significantly (P<0.05) decreased total volatile fatty acids (TVFA's) concentration and fungi counts comparing to the control. Total bacteria counts was significantly (P<0.05) increased with increasing the level adding of LOG. On the other hand, inclusion LOG at different levels had no significant effect on both actinomyces and cellulolytic bacteria counts. Adding LOG decreased total aerobic bacterial counts (TABC), total Coliform counts (TCC), feacal coliform counts (FCC), total fungi counts (TFC) and total entero bacteriacea counts (TEBC), meanwhile, total yeast count (TYC) was increased. It can be mentioned that adding nature bioactive mixture (LOG) in drinking water of Rex rabbits occurred an improving in their growth performance, decreasing mortality rate, decreasing both blood total cholesterol and blood triglyceride. Also, it had no adverse effect on carcass characteristics or ceacum parameters and improves the microbial activity. The best levels of adding from LOG was 20 ml LOG/ liter dinking water

Keywords: Bioactive mixture, rabbits, growth performance, carcass characteristics, blood constituents, ceacum and microbiological parameters.

INTRODUCTION

The European ban on the non-therapeutic use of antibiotic growth promoters and limits on the use of other drugs have increased digestive disorders and mortality in growing rabbits. In addition, consumers demand natural products, and therefore synthetic active compounds should be replaced by natural ones (Dalle Zotte et al., 2016).

Also, Dalle Zotte et al., (2016) noted that plants (whole plants, leaves or seeds, mainly used as feedstuffs) and their extracts (considered as additives) are being increasingly used in animal nutrition as appetisers, digestive and physiological stimulants, colorants, and antioxidants, and for the prevention and treatment of certain pathological conditions.

The world health organization (WHO) encourages the acceptance of feed additives (Line-Eric et al., 1998 and Aboul-Fotouh et al., 1999).

Feed additives are a group of nutrient that helps in improving the efficiency of feed utilization and thus reducing the high cost of feed. In the past, antibiotics were the most routinely used feed additives. However, nowadays use of antibiotics is not only limited but their use in livestock and poultry industry also have been banned in many countries due to the reasons like alteration of natural gut microbiota and drug resistance in bacteria and humans. As a result, to replace them without adversely affecting the performance of birds, natural growth promoters such as prebiotics, probiotics, synbiotics, enzymes, plant extracts, etc., can be used to feed the broilers (Borazjanizadeh et al., 2011).

Also, in the recent years, noticed that using bioactive feed additives become important materials used to improve the efficiency of feed utilization and growth performance of calves (Aiad et al., 2008; Ahmed et al., 2009).

Medicinal herbs such as garlic, onion and have been reported to possess antibacterial, antiseptic, anti-inflammatory, antiparasitic and immunomodulatory properties (Muhammad et al., 2009).

Natural additives such as lemon, onion or garlic to food will increase the antioxidant content and may have potential as a natural antioxidant and thus inhibit unwanted oxidation processes (Wangensteen et al., 2004). Adding garlic to food encouraged stimulation of immune function, enhanced foreign compound detoxification and resistance various stresses (Amagase et al., 2001).

Most of the plant parts contain compounds with proven antibacterial, antiviral, antiparasitic, antifungal properties have antihypertensive, hypoglycemic, antithrombotic, antihyperlipidemic, anti inflammatory and antioxidant activity (Lampe 1999).

Garlic rich in sulphur compound such as cysteine sulfoxides (Lancaster and Shaw 1989). Also, it contained steroidal glycosides, lectins, prostaglandins, fructan, pectin, essential oil, adenosine, vitamins B_1, B_2, B_6, C and E, biotin, nicotinic acid, fatty acids, glycolipids, phospholipids, anthocyanins, flavonoids, phenolics and amino acids (Matsuura et al., 1988; Kaku et al., 1992).

Garlic as natural growth promoters can be potential alternatives for common artificial growth promoters like antibiotics (Demir et al., 2003).

Garlic improves the performance of broilers when added at the rate of 1% of broiler ration and can be a viable alternative to antibiotic growth promoter in the feeding of broiler chicken (Karangiya et al., 2016).

Onion bulbs possess numerous organic sulphur compounds including Trans-S-(1-propenyl) cysteine sulfoxide, S-methyl-cysteine sulfoxide, spropylcycteine sulfoxides and cycloallicin, flavinoids, phenolic acids, sterols including cholesterol, stigma sterol, b-sitosterol, saponins, sugars and a trace of volatile oil compounds mainly of sulphur compounds (Melvin et al., 2009).

Lemon is a good source of potassium (145 mg per 100 g fruit), bioflavonoid, and vitamin C (40 to 50 mg per 100 g), this equal twice time as much as oranges (Hassan and Abdel-Raheem, 2013)

(Ahmed et al., 2016) noted that average daily gain was improved by 20%, 29%, 36.1% and 19.3% when rabbits fed diets contained 5, 10, 15 and 20 ml LOG / kg feed, respectively comparing with the control group.

(Senthilkumar et al., 2015) reported that garlic supplementation had no significant effects on major carcass components and organ characteristics. Also, (Raeesi et al., 2010) noted that supplementation of 1% and 3% garlic in the broiler diet had no significant effects on relative weights of carcass, fat pad, or digestive organs among different treatments. Furthermore, Nobakht (2013) indicated that dried lemon pulp improved growth performance of chickens across the entire production period.

On the other hand, serum cholesterol was significantly decreased by dietary dehydrated onion in experimentally hypercholesterolemic rats (Vidyavati et al., 2010).

So, the present work aimed to study the impact of adding bioactive natural mixture composed of lemon, onion and garlic juice (LOG) at different levels in drinking water of Rex Rabbits on productive and reproductive performance, carcass characteristics, blood constituents and micobiol activity.

MATERIALS AND METHODS

Animals and feeds

This study was carried out in co-operation work among Animal Production Department, National Research Centre, 33 El-Bohouth Street, P.O: 12622, Dokki, Giza, Egypt and Regional Center for Food and Feed, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt.

The present work aimed to investigate the impact of adding natural bioactive mixture composed of lemon, onion and garlic (LOG) juice at portions (1.00: 1.00: 0.125/ liter clean water), respectively on productive performance of Rex rabbits.

The natural bioactive mixture was prepared as the following:

- Fresh garlic bulbs, onion, and limon were obtained from the local market and cut into small pieces.
- About 250 ml of distilled water per 100 g of peeled onion/ or garlic and limon were added and crushed in a mixing machine in the presence of some crushed ice.
- The resultant slurry of every one was squeezed and filtered 3 times through a fine cloth.
- The aqueous extract of 3 materials was stored in dark brown bottle with scrow cap at 4 °C until use.

A total number of three hundred Rex rabbits aged 4 to 5 weeks (772±22 g) were divided into four equal comparable experimental groups (75 in each). Feeding trial lasted for 8 weeks (56 days) and the first experimental group rabbits received the basal diet that formulated to cover the requirements of growing rabbits according to NRC (1977) recommendations and considered as (control group), meanwhile, drinking water was offered ad libtum without containing any additives (Zero LOG). On the other hand, the 2nd; 3rd and 4th groups were also fed the basal diet; meanwhile drinking water was added at levels 10, 20 and 30 ml LOG/ liter of drinking water for the 2nd; 3rd and 4th groups, respectively.

The experimental work was carried out in privet industrial rabbitry located near Skarah City, Giza Province, Egypt, during the period from May to July. Rabbits were raised in semi-closed rabbitry with wire-netted windows on their sides for providing natural ventilation. The windows were oriented with an elevation of 2 meters from the floor. The floor was made from ceramic plates and has moderately slope (from the middle to both sides) to facilitate water drainage towards a large longitudinal gutter outside the rabbitry.

Feed intake (FI) and live body weight (LBW) values were recorded through out the experimental growing period that lasted for 56 days to calculate the daily feed intake (DFI), average daily gain (ADG), feed conversion (FC) and mortality rate.

Carcass traits

At the end of the feeding trial ten representative rabbits from each treatment were randomly chosen to determine the carcass parameters according to (Blasco *et al.,* 1993). Rabbits were fasted for 12 hours before slaughter (Abd El-Monem 1995) which was performed according to the Islamic rules. Animals were weighed just before slaughter, slaughter weight (SW) was recorded and as well as after complete bleeding. Warm carcass weight and edible offal's (Giblets) includes heart, liver, kidneys, lungs, spleen and testes were individually weighed and recorded.

Dressing percentages (DP)

Dressing percentages (DP) were calculated according to the following equation (DP = CW / SW). On the other hand, absolute and relative giblets weight was also calculated as described by Seleem et al., (2003).

Ceacal parameters

Five caecal samples per treatment were separated carefully used to estimate the caecal pH, caecal total volatile fatty acids (TVFA's) concentration and caecal microbial counts.

Caecal pH was immediately determined using digital pH meter; while caecal total volatile fatty acids (TVFA's) concentrations were determined according to AOAC (2005) methods.

The serial dilution plate count procedure was used to estimate the total number of different groups of micro-organisms, namely, bacteria; fungi and actinomycetes. Three selective media were used for plate count. These were; nutrient agar (Difco, 1966) and Martin's medium (Allen, 1953) for fungi and glucose asparagine agar for actinomycetes. Plates were incubated at 28 °C for mesophilic. Fungal colonies were counted after 3 days; bacteria and actinomycetes colonies after 10 days and colonies of actinomycetes have been distinguished by their characteristic growth, and by the use of straight needle and microscopic examination. Counts were presented per one g oven dry weight of material.

Growth microbial strains determinations

Samples of water in selective media were applied as follows:

Determination of total aerobic bacterial Counts (TABC)

Aerobicbacterial counts were estimated on glucose yeast extract nutrient agar medium as the method reported by **(APHA, 2005)** using pouring plate technique. Suitable plates were counted after incubation at 37°C for 48 hours.

Determination of total coliform and fecal coliform counts (TCC)

Coliform and feacal coliform counts were estimated on MacConkey agar (1905) using pouring plate technique. Suitable plates were counted after 24 hours at 37°C and 44.5°C for total coliform and feacal coliform counts, respectively.

Determination of total yeast counts

Total counts of yeast were determined in a ruse-bengle chloramphenicol agar according the methods described in (Oxpoid Manual 2000). Plates were incubated at 22-25°C for 7 days.

Determination of total fungi counts

Total counts of fungi were determined on petato dextrose agar (PDA) medium (Christensen, 1957).

Total Entero-bacteriacea Counts (TEBC)

Violetred bile glucose agar medium plates (European Directorate for the Quality of Medicines and Healthcare, 2008) were incubated with 1 ml of the appropriate dilutions andincubated overnight at 37°C. After incubatation on clearly visible purple colonies surrounded by a purple halo were estimated as entero bacteriaceae counts (Difco, 2000).

Analytical procedures

The different analysese were conducted in laboratories of Animal Production Departement, National Research Centre and Regional Center for Food and Feed.

Blood samples were collected from the slaughtered rabbits and centrifuged at 5000 r.p.m for 20 min., for preparation of blood plasma. Plasma was kept frozen at -18 °C for subsequent analysis. Plasma total protein was determined according to Armstrong and Carr (1964) and Witt and Trendelenburg (1982); albumin according to (Doumas et al., 1971) and (Tietz, 1986); triglycerides (Fossati and Principe, 1982); total lipids (Postma and Stroes, 1968); total cholesterol according to (Allain et al., 1974) and (Pisani et al., 1995); plasma Glutamic Oxaloacetic Transaminase (GOT) and Glutamic Pyruvic Transaminase (GPT) activities were determined as described by Reitman and Frankel (1957) and Harold (1975); high-density lipoprotein concentration (HDL-C) was estimated according to the Assmann (1979) using commercial kits; red blood cell (RBC's) & white blood cell counts (WBC's) according to Weiss and Wardrop (2010); hemoglobin (Hb) concentration according to (Bunn, 2011; Elghetany and Banki, 2011); urea according to (Patton and Crouch, 1977). On the other hand, globulin and albumin: globulin ratio (A: G ratio) were calculated.

Also, mortality rate was estimated through out growing period at different stages.

Both composition and calculated chemical analysis of the pelleted basal diet was presented in (Table 1).

Table: 1. composition and calculated chemical analysis of the basal diet.

Ingredients	Kg/ ton
Clover hay	405.00
Wheat bran	250.00
Yellow corn	140.00
Soybean meal (44%)	110.00
Molasses	30.00

Vinas **Bone meal** **Lime stone** **Sodium chloride** **Vitamins & Mineral Premix*** **DL-Methionine**	30.00 17.50 7.00 5.50 3.50 1.50
Calculated chemical composition **	
Crude protein (CP) **Crude fiber (CF)%** **Ether extract (EE)** **Digestible energy (Kcal/Kg DM)**	18.00 14.00 3.00 2720

* Vitamins & Mineral Premix: Vitamins & Minerals premix per Kilogram contained Vit.A (10,000 IU), Vit.D_3 (9000 IU), Vit.E (10000 IU), Vit.K (3 IU), Vit.B_1 (2 IU), Vit.B_2 (6 IU), Vit.B_6 (2 IU), , Biotin (0.2 mg), Choline (1200 mg), Niacine (40 mg), Zinc. (60 mg), Cu. (0.1 mg), Mn. (85 mg), Fe. (75 mg), Folic acid (5 mg) and Pantothenic acid (20 mg).
** Calculated chemical composition: calculated according to NRC (1977) for rabbits.

Statistical analyses

Data collected were subjected to statistical analysis as one way analysis of variance according to SPSS (2008). Duncan's Multiple Range Test Duncan, (1955) was used to separate means when the dietary treatment effect was significant according to the following model:

Yij = μ + Ti + eij   W here: Yij = observation.
μ = overall mean.

Ti = effect of treatment for i = 1–4, 1 = first ^{1st}group (control) fed commercial diet that no contained LOG in drinking water), 2 = second 2^{nd} group fed commercial diet and adding 10 ml LOG/ litter drinking water), 3 = third 3^{rd} group fed commercial diet and adding 20 ml LOG/ litter drinking water) and 4 = fourth 4^{th} group fed commercial diet and adding 30 ml LOG / litter drinking water).

eij = the experimental error.

Percentage values were transformed to Arc-Sin values to approximate normal distribution before being statistically analyzed. Duncan's new multiple range tests was used to test the significance of the differences among means (Duncan, 1955).

Growth performance

Live body weight of the experimental group rabbits

Data of live body weight that presented in Table (2) cleared that inclusion LOG at different levels (10, 20 and 30 ml LOG/ liter drinking water) caused significantly (P<0.05) increasing in live body weight (LBW) through out the 2nd weeks, 4th weeks, 6th weeks and 8th weeks of growing period in comparison with the control (Zero LOG). Also, average daily gain (ADG) was significantly (P<0.05) increased with adding LOG at different levels mentioned above comparing to control. The corresponding values of ADG were 25.18, 28.63, 31.38 and 30.77 g for D1, D2, D3 and D4, respectively. These increasing in ADG were reaching to 13.7, 24.6 and 22.2% for D2, D3 and D4, respectively compared to control one. Rabbits that received 20 ml LOG/ liter drinking water recorded the best value of ADG followed by 30 and 10 ml LOG/ liter drinking water.

These results in agreement with those found by (Ahmed et al., 2016) who noted that average daily gain was improved by 20, 29, 36.1% and 19.3% for rabbits fed (5, 10, 15 and 20 ml LOG/ kg feed, respectively) compared to the control group (0 ml LOG/ kg feed). On the other hand, Onion and garlic caused an improvement in growth this reason related to increase the inflow of glucose into tissues and thyroid like activity (Habbak et al., 1989). In addition to, improvement of live body weight gain by feeding animals on onion and/or garlic may be due to their contents of sulphar compounds that are considered as active antimicrobial agents and improve immunity (Dedi and Elssenuwenger 2000; Ibrahiem et al., 2004).

Feed intake of the experimental group rabbits

Daily dry matter intake (DMI) was not affected by adding LOG at different levels, the average daily DMI was varied from 94 to 98 g/head/day among the different experimental group rabbits (Table 2). These results might explain that adding bioactive mixture (LOG) at different experimental levels had no adverse effect on rabbit's palatability.

RESULTS AND DISCUSSION

Table: 2. Growth performance of the experimental group rabbits drinking water containing different levels of LOG.

Item	Duration period	Experimental diets			
		Control	10 ml LOG/ liter	20 ml LOG/ liter	30 ml LOG/

		Zero LOG (D_1)	drinking water (D_2)	drinking water (D_3)	liter drinking water (D_4)
Rabbits number in each group		75	75	75	75
1- Live body weight, g					
Initial weight		772±21.4	777±19.3	770±25.2	768±20.4
Live body Weight (LBW), g at	**2nd weeks**	1112^c±32.4	1151^b±29.6	1180^a±33.4	1172^a±31.2
	4th weeks	1488^c±34.2	1583^b±33.6	1690^a±42.5	1660^a±40.3
	6th weeks	1818^c±40.4	1985^b±39.6	2082^a±40.8	2058^a±43.4
	8th weeks	2228^c±43.2	2471^b±44.4	2570^a±43.7	2540^a±42.5
Average daily gain (ADG), g at	**2nd weeks**	24.29^c±1.23	26.71^b±1.82	29.29^a±1.54	28.88^a±1.87
	4th weeks	25.57^c±1.11	28.79^b±1.03	32.85^a±1.64	31.85^a±1.91
	6th weeks	24.90^c±1.05	28.76^b±1.72	31.24^a±1.58	30.71^a±1.14
	8th weeks	26.00^c±1.44	30.25^b±1.97	32.14^a±2.07	31.64^a±1.99
Means ± SE		**25.19^c±1.21**	**28.63^b±1.64**	**31.38^a±1.71**	**30.77^a±1.73**
2- Feed intake					
Daily dry matter intake (DMI), g	2nd weeks	87.00±3.43	88.00±3.36	90.00±3.64	92.00±3.46
	4th weeks	91.00±4.12	94.00±4.28	97.00±4.42	98.00±4.37
	6th weeks	95.00±3.28	97.00±3.52	99.00±3.64	97.00±3.55
	8th weeks	10.3.00±6.17	105.00±6.08	106.00±6.24	105.00±6.33
Means ± SE		**94.00±4.25**	**96.00±4.31**	**98.00±4.49**	**98.00±4.43**
3- Feed conversion expressed as					
g of dry matter intake/ g gain	**2nd weeks**	3.58^c±0.13	3.29^b±0.11	3.07^a±0.13	3.19^{ab}±0.14
	4th weeks	3.56^c±0.18	3.27^b±0.20	2.95^a±0.18	3.05^a±0.17
	6th weeks	3.82^c±0.16	3.37^b±0.19	3.17^a±0.20	3.16^a±0.22
	8th weeks	3.96^c±0.20	3.47^b±0.18	3.29^a±0.21	3.32^a±0.23
Means ± SE		**3.73^c±0.17**	**3.35^b±0.17**	**3.12^a±0.18**	**3.18^a±0.19**

a, b, and c: Means in the same row having different superscripts differ significantly (P<0.05).
LOG: Natural bioactive mixture juice composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ litter clean water

Table: 3. Mortality rate of the experimental group rabbits drinking water containing different levels of LOG.

Item	Duration period	Experimental diets			
		Control Zero LOG (D_1)	10 ml LOG/ liter drinking water (D_2)	20 ml LOG/ liter drinking water (D_3)	30 ml LOG/ liter drinking water (D_4)
Rabbits number in each group		75	75	75	75
Number of live Rabbits at the end of	**2nd weeks**	68	70	71	72
	4th weeks	65	67	69	70
	6th weeks	62	66	68	69
	8th weeks	60	65	68	69
Total dead rabbits over the entire period		15	10	7	6
Mortality rates (%) during	2nd weeks	9.33^a	6.67^b	5.33^c	4.00^d
	4th weeks	4.41^a	4.29^b	2.82^c	2.78^c
	6th weeks	4.62^a	1.49^b	1.45^c	1.43^c
	8th weeks	3.23^a	1.52^b	0.00^c	0.00^c
Mortality rate over the entire period		20.00^a	13.33^b	9.33^c	8.00^d

a, bc and d: Means in the same row having different superscripts differ significantly (P<0.05).
LOG: Natural bioactive mixture juice composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ litter clean water.

These results were in harmony with those noted by (Omer et al., 2015) and Ahmed et al., (2016) who reported that incorpration LOG at (0, 5, 10 15 and 20 ml/ kg feed) had no significant effect on DM intake and DM intake ranged from 106 to 112 g/head/day.

Feed conversion of the experimental group rabbits

Data of feed conversion mentioned that adding LOG at different levels in drinking water of rabbits (10, 20 and 30 ml LOG/ liter drinking water) occured an improving in feed conversion that expressed as (g of dray matter intake/ g gain) through out the 1st week, 2nd week, 3rd week and 4th week of growing period comparing with the control

(Zero LOG). In addition for that, the best feed conversion was recorded by adding 20 ml LOG (3.12 g DMI/ g gain) followed by 30 ml LOG (3.18 g DMI/ g gain) and 10 ml LOG (3.35 g DMI/ g gain), meanwhile control (zero LOG) recorded (3.73 g DMI/ g gain). These results might be due to the effective to improve immunity and decrease debility incidence, which agree with the findings of Aboul-Fotouh et al., (1999); Nadi (1999); Aboul-Fotouh et al., (2000); Ahmed et al., (2009) who reported that nutrition plays important role in diminishing growth rate. On the other hand, Aiad et al.., (2008) noted that feeding suckling buffalo calves diets supplemented with 2.5, 5 or 7.5% bioactive natural mixture composed of lemon, onion and garlic juice (LOG), feed conversion that expressed as kg DM, TDN and DCP/ kg gain improved by 31.05, 36.64 and 21.75%, respectively in comparison with the control. Also, Eid et al.,(2010) noted that feeding growing Californian rabbits ration contained 0.50% green tea significantly (P<0.05) improved feed conversion compared to control ration, corresponding values were (3.07 vs. 3.21 g feed intake/g gain) for 0.50% green tea and control rations, respectively.

Mortality rate of the experimental group rabbits

Data of Table (3) cleared that mortality rate was decreased with increasing the level of LOG addition at 2st week, 4nd week, 6rd week and 8th week, in addition to mortality rate over the entire period.

These results were in agreement with those noted by Badr et al., (2013) who recorded that when rabbits received drinking water supplemented with 1, 2 and 3 ml of natural mixture juice (NMJ) of garlic, onion and lemon/ Liter filter drinking water caused significantly (P<0.05) decreasing in total mortality rate (18.18, 12.73 and 10.91 %) in comparison with control group (21.82%). Also, Omer et al., (2016) reported that mortality rate was decreased with increasing the level of LOG that added to broiler chick rations through out over the entire period. Also, they noticed that the corresponding values of mortality rate were 5.77, 5.77, 3.48 and 1.92% for broiler chicks received diets containing 0, 10, 20 and 30 ml LOG/ kg feed, respectively.

Also, during the post weaning phase, (Habeeb et al., 1997) estimated the mortality rate at 18% in the summer, while no mortality was recorded during the winter. During 4-5 weeks of age, mortality rate percentage was significantly lower in spring than in winter and autumn (El-Desoki, 1991). During 5-9, 9-13 and 5-13 weeks of age intervals, mortality rate values were higher in summer and lower in spring than in autumn and winter (Shehata et al., 1998). However, El-Maghawry (1993) found that the maximum mortality was recorded during spring, while the minimum mortality was recorded in autumn.

Carcass characteristics of the experimental group rabbits

Data of Table (4) cleared that inclusion LOG at different levels (10, 20 and 30 ml LOG/ liter drinking water) significantly increased both carcass weight (CW) and dressing percentages (DP). This improving may be related to LOG addition or also for the difference in slaughter weight as shown from the results obtained.

These results in agreement with those found by Badr et al., (2013) who noted that the differences in carcass weight traits as a results to the effect of adding natural juices of garlic and onion and lemon mixture to rabbit diets were significant (P<0.05). Also, they noticed that the highest values for dressing percentages was recorded with rabbits received 2 or 3 ml/L./natural mixture juice (NMJ) in drinking filter water compared to those had (1 ml/L NMJ) or the control (0 ml/L NMJ). On the other hand Omer et al., (2015) reported that inclusion LOG at (0, 5, 10, 15 and 20 ml LOG/ kg feed) in rabbit rations had no significant effect on dressing percentage, however adding 5ml LOG/kg feed recorded the best values of dressing percentages compared to the other groups. This may be related to superior of rabbits received 5 ml LOG / kg feed in carcass weight comparing with the other groups. Also, they noted that adding LOG mixture caused an in significant increasing in values of carcass weight.

Furthermore, Eid et al., (2010) noted that feeding growing Californian rabbits ration contained 0.50% green tea had no significant effect on dressing percentages. Meanwhile Cardinali et al., (2015) noted that feeding New Zealand White rabbits on rations contained 0.2% oregano (Origanum vulgare) aqueous extract or 0.1% oregano extract +0.1% rosemary extract significantly (P<0.05) increased both carcass weight and carcass yield percentages. In addition to, Omer et al., (2010) recorded that dressing percentages that calculated as carcass weight/ slaughter weight (CW/SW) was not affected by addition 0.5% lemongrass or active dried yeast to rabbit's diet compared to control diet. However, they noticed that adding 0.5% lemongrass significantly (P<0.05) increase dressing percentages as carcass weight/ empty body weight

(CW/ EBW) compared to active dried yeast and control diets. Also, Omer et al., (2012) noted that feeding rabbit's diet containing 1.5% mixture of some medicinal plants composed of (Lupinus albus L, Trigonella foenum-graecum L and Cassia senna L) as feed additives had no significant effect on carcass weight and dressing percentages of rabbits.

Also, Omer et al., (2013) noted that adding herbal mixture formulation consisting of fennel *(Foeniculum vulgare)* seeds or oregano leaves (*Origanum vulgare* L.) and mixture of them had no significant effect on carcass weight and dressing percentages of rabbits.

In addition to, data of Table (4) showed that incorporation LOG at 20 and 30ml/ liter drinking water significantly (P<0.05) increased absolute and relative values of edible offals (giblets) that includes liver, heart, kidneys, testes spleen and lungs compared to control. Meanwhile adding 10 ml LOG/ liter drinking water in significant increased the same values mentioned above in comparison with the control. These results not agreement with those found by Omer et al*.,* (2015) who noted that adding LOG at (0, 5, 10, 15 and 20 ml/ kg feed of New Zealand White rabbits had no significant effect giblets weights that included (liver, heart, kidneys, spleen and tests). However, they observed that inclusion LOG mixture in significantly increased liver and kidney weights up to 15 ml LOG/ kg feed compared to control, while adding 20 ml LOG/ kg feed in significantly decreased both liver and kidney weights. Also, Lambertini et al., (2004) noticed that feeding rabbits on rations contained non-enriched yeast or 0.400 mg/kg chromium-enriched yeast had no significant (P>0.05) effect on liver and kidneys that expressed as % of carcass weight. Also, Bonomi et al. (1999) not found any effect on the weight of the main organs for rabbits received chromium-yeast. Also, Omer et al., (2010) mentioned that addition 0.5% lemongrass or active dried yeast to rabbit diets had no significant effect on internal offals (giblets) weight. Also, Ahemen et al. (2013) showed that internal organ weights of the male rabbits no affected by dietary treatment levels (0, 5, 10 and 15% water spinach meal). In contrast, the(Eid et al., 2010) noted that feeding growing Californian rabbits ration contained 0.50% green tea significantly (P<0.05) decreased liver, heart and kidneys weight.

Blood parameters of the experimental group rabbits

Data illustrated in Table (5) cleared that with increasing level of adding LOG in drinking water values of red blood cells, white blood cells, hemoglobin, total protein, Albumin, globulin and albumin: globulin ratio were significantly (P<0.05) increased.

These results are in agreement with those established by Hassan and Abdel-Raheem (2013) who mentioned that calves fed diets contained garlic had greater (P<0.05) serum concentrations of globulin. Mean while the present results were not agreement with those obtained by Omer *et al.* (2015) who noted that, when rabbits received diets contained different levels of bioactive mixture juice (0, 5, 10, 15 and 20 ml LOG/ kg feed) the dietary treatments had no significant effect on total protein and globulin. Also, Ahmed et al., (2009) reported that serum total protein were not significantly effect by the natural additive (LOG) when added to calves rations. On the other hand, Bush (1991) found a positive correlation between dietary protein and plasma protein concentration. This indicates that the supplement had not affect protein synthesis in liver function. Also, the low level of proteins may be attributed to a decrease in the protein absorbed and synthesized and an increase in protein losses.

On the other hand, as presented in (Table 5) the present results mentioned that values of blood NH_3, blood urea, GOT, GPT, total lipids, phospholipids, triglycerides, HDL-cholesterol and total cholesterol were significantly (P<0.05) decreased with increasing levels of LOG incorporated in drinking water of rabbits. These results were in harmony with those obtained by (Omer et al., 2015) who noted that values of triglycerides of rabbits was significantly (P<0.05) decreased comparing to control. Also, they reported that incorporation LOG in rabbit rations at 15ml or 20ml/ kg DM feed significantly (P<0.05) decreased total cholesterol in comparison with the control. But, addition 5 ml or 10 ml LOG / kg DM not significant altered comparing with the other groups. Also, these results are in agreement with those noted by Prasad et al., (1982); (Lau et al., 1987); (El-Hosseiny et al., 2000) and(Ahmed et al., 2009)

Table: 4. Dressing percentages and edible offals (giblets) weight of the experimental group rabbits drinking water containing different levels of LOG.

Item	Experimental diets

		Control Zero LOG (D_1)	10 ml LOG/ liter drinking water (D_2)	20 ml LOG/ liter drinking water (D_3)	30 ml LOG/ liter drinking water (D_4)
Rabbits number in each group		10	10	10	10
Slaughter weiight (SW), g		2228^{c}±42.6	2471^{b}±41.8	2570^{a}±45.5	2540^{a}±48.4
Carcass weight (CW)		1299^{c}±26.3	1552^{b}±27.4	1661^{a}±26.6	1630^{a}±28.3
Dressing percentages (DP)		58.30^{c}±1.03	62.81^{b}±1.05	64.63^{a}±1.18	64.17^{a}±1.11
Liver weight	**Absolute, g**	57.28^{b}±2.31	68.40^{ab}±2.43	72.59^{a}±2.87	75.98^{a}±2.94
	Relative (%)	2.57^{c}±0.10	2.77^{bc}±0.11	2.82^{b}±0.11	2.99^{a}±0.10
Heart weight	**Absolute, g**	10.02^{b}±0.77	11.40^{ab}±0.75	12.56^{a}±0.88	12.21^{a}±0.93
	Relative (%)	0.45^{b}±0.014	0.46^{ab}±0.03	0.49^{a}±0.02	0.48^{a}±0.02
Kidneys weight	**Absolute, g**	20.00^{b}±0.88	21.38^{ab}±0.91	22.34^{a}±0.99	21.71^{a}±0.87
	Relative (%)	0.90^{a}±0.002	0.87^{a}±0.001	0.87^{a}±0.001	0.85^{b}±0.001
Testes weight	**Absolute, g**	7.00^{b}±0.09	7.00^{b}±0.08	8.50^{a}±0.10	800^{a}±0.10
	Relative (%)	0.40^{a}±0.001	0.28^{c}±0.002	0.33^{ab}±0.001	0.31^{b}±0.001
Spleen weight	**Absolute, g**	1.80^{c}±0.13	2.50^{b}±0.02	2.79^{a}±0.02	2.71^{a}±0.02
	Relative (%)	0.08^{b}±0.01	0.10^{ab}±0.01	0.11^{a}±0.01	0.11^{a}±0.01
Lungs weight	**Absolute, g**	11.50^{b}±0.91	12.83^{ab}±0.85	14.00^{a}±0.90	14.93^{a}±0.88
	Relative (%)	0.52^{b}±0.04	0.52^{ab}±0.01	0.54^{a}±0.02	0.59^{a}±0.02

a, b and c: Means in the same row having different superscripts differ significantly (P<0.05).
LOG: Natural bioactive mixture juice composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ litter clean water.
Dressing percentages (DP) = carcass weight (CW) * 100 / slaughter weiight (SW).

Table: 5. Blood parameters of the experimental group rabbits drinking water containing different levels of LOG.

Item	Experimental diets			
	Control Zero LOG (D_1)	10 ml LOG/ liter drinking water (D_2)	20 ml LOG/ liter drinking water (D_3)	30 ml LOG/ liter drinking water (D_4)
Red blood cells (N x 10^6 /mm^3)	5.52^{c}±0.26	6.14^{b}±0.30	7.07^{a}±0.32	7.46^{a}±0.29
White blood cells (Nx0^3/mm^3)	6.20^{c}±0.24	7.05^{b}±0.23	8.01^{a}±0.29	8.57^{a}±0.34
Hemoglobin (mg/ dl)	9.57^{d}±0.34	11.69^{c}±0.37	12.53^{b}±0.41	13.67^{a}±0.53
Total protein (mg/ 100ml)	5.16^{d}±0.18	6.09^{c}±0.23	7.05^{b}±0.21	7.95^{a}±0.24
Albumin (mg/ 100ml)	3.07^{d}±0.13	3.89^{c}±0.17	4.67^{b}±0.17	5.28^{a}±0.15
Globulin (mg/ 100ml)	2.09^{d}±0.13	2.20^{c} ±0.17	2.38^{b}±0.17	2.67^{a}±0.15
Albumin: globulin ratio	1.47^{c}±0.03	1.77^{b}±0.05	1.96^{a}±0.08	1.98^{a}±0.13
Blood NH_3 (µg/ ml)	5.07^{a}±0.26	4.19^{bc}±0.24	4.07^{c}±0.30	3.72^{c}±0.28
Blood Urea N (mg/ dl)	16.74^{a}±1.29	13.29^{b}±1.02	12.75^{bc}±1.1	11.46^{c}±0.79
GOT (U/ L)	35.84^{a}±1.23	33.66^{b}±1.28	32.96^{bc}±1.17	30.41^{c}±1.42
GPT (U/ L)	25.27^{a}±0.54	23.53^{b}±0.63	22.72^{bc}±0.84	20.81^{c}±0.75
Total lipids (µg/ ml)	195.7^{a}±4.37	176.3^{b}±3.89	172.1^{bc}±3.11	167.9^{c}±2.67
Phospholipids (µg/ ml)	1.83^{a}± 0.04	1.75^{b}±0.02	1.59^{c}±0.04	1.51^{c}±0.04
Triglycerides (µg/ ml)	449.6^{a}±9.52	431.8^{b}±6.87	424.7^{bc}±4.88	419.5^{c}±4.51
HDL-cholesterol (µg/ ml)	11.86^{a}±0.64	10.63^{b}±0.56	9.84^{b}±0.43	9.11^{c}±0.29
Total cholesterol (µg/ ml)	151.6^{a}±3.84	142.5^{b}±2.97	132.6^{c}±2.85	126.9^{d}±2.85

a, b, c and d: Means in the same row having different superscripts differ significantly (P<0.05).
LOG: Natural bioactive mixture juice composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ litter clean water.
HDL: High deniseyt lipoprotein

who noticed that additive garlic occurred lowering in total lipids and high values of the blood components in calves and small ruminants. Also, our results are in agreement with those established by Hassan and Abdel-Raheem (2013) who recorded that calves fed diets contained garlic caused lowering in cholesterol content compared to those fed the control diet. Also, El-(Ashry et al., 2006); (Abo El-Nor et al., 2007) and Ahmed et al., (2009) found that the mean values of blood metabolites were higher in animals fed diets containing medicinal herbs than control. The lowering in total cholesterol in present study could be ascribed to LOG which is thought to have various pharmacologic properties. For example, it has been found to lower serum and liver cholesterol (Qureshi et al., 1983), inhibit bacterial growth (Cavallito and Bailey 1994), inhibit platelet growth and reduce oxidative stress (Horie et al., 1992). Meanwhile, the present results were disagreement with those found by Omer et al. (2015) who showed that values of blood total lipids of rabbits was significantly (P<0.05) increased with increasing level of addition from LOG (10, 15 and 20 ml/ LOG/ kg feed). Also, they noticed that high density lipoprotein (HDL) was not affected by inclusion LOG at (0, 5, 10, 15 and 20 ml LOG/ kg feed) in rabbits.

Caecal pH, total volatile fatty acids and microbiological parameters

Rabbit is a small non-ruminant herbivore and it feeding in more similar to ruminant feeding than to poultry feeding as rabbit digestive physiology shows some similarity to ruminant, particularly ceacal processing of fiber (Marounnek et al., 2000). The ceacum plays a key role in the digestive physiology as the major sit of fermentation (fiber degradation, etc…).

Fermentation pattern in rabbit ceacum resemble that of the rumen, however it shows lower fibrocystic microbial activity and relatively higher analytic and proteolytic microbial activity (Gidenne, 1997).

Table: 6. Caecal parameters of the experimental group rabbits drinking water containing different levels of LOG.

Item	Experimental diets			
	Control Zero LOG (D_1)	10 ml LOG/ liter drinking water (D_2)	20 ml LOG/ liter drinking water (D_3)	30 ml LOG/ liter drinking water (D_4)
Caecal pH	6.55^{a}±0.05	6.53^{a} ±0.04	6.51^{ab}±0.05	6.49^{b}±0.04
Total volatile fatty acids (TVFA's)	6.42^{a}±0.52	4.18^{b}±0.49	4.00^{bc}±0.46	3.85^{c}±0.42
Bacteria counts (10^5)	40.33^{c}±1.85	48.67^{b}±1.77	52.45^{a}±1.66	54.67^{a}±1.70
Fungi counts (10^3)	5.85^{a}±0.53	5.31^{b}±0.50	5.27^{bc}±0.48	5.12^{c}±0.51
Actinomyces counts (10^3)	4.67±0.20	4.50±0.19	4.98±0.17	4.48±0.18
Cellulolytic bacteria counts (10^3)	25.20±0.52	25.22±0.55	25.27±0.51	25.30±0.50

a, b and c: Means in the same row having different superscripts differ significantly (P<0.05).

Table 7. Growth microbial strains of drinking water containing different levels of LOG.

Item	Experimental levels			
	Zero ml LOG/ liter drinking water	10 ml LOG/ liter drinking water	20 ml LOG/ liter drinking water	30 ml LOG/ liter drinking water
Total aerobic bacterial counts (TABC)	$36x10^{-3}$	$25x10^{-3}$	$17X10^{-3}$	$12x10^{-3}$
Total Coliform counts (TCC)	$70x10^{-2}$	$52x10^{-2}$	$35X10^{-3}$	$22x10^{-3}$
Feacal coliform counts (FCC)	$53x10^{-2}$	$40x10^{-2}$	$25X10^{-2}$	$17x10^{-2}$
Total yeast count (TYC)	$23x10^{-2}$	$30x10^{-2}$	$33x10^{-2}$	$38x10^{-2}$
Total fungi counts (TFC)	$6x10^{-5}$	$3x10^{-5}$	$2x10^{-5}$	$0x10^{-5}$
Total entero bacteriacea counts (TEBC)	+	+	-	-

As presented in Table (6) the results cleared that caecal pH values that ranged from 6.49 to 6.55 was insignificantly decreased with adding LOG in drinking water up to 20 ml LOG/ liter water, meanwhile, incorporation 30 ml/ liter water significantly decreased their value of caecal pH in comparison with the control. Meanwhile, adding LOG at different levels significantly ($P<0.05$) decreased total volatile fatty acids (TVFA's) concentration and fungi counts comparing to the control. However, total bacteria counts was significantly ($P<0.05$) increased with increasing the level adding of LOG in drinking water. On the other hand, inclusion LOG at different levels had no significant effect on both actinomyces and cellulolytic bacteria counts. Abdel-Hakim et al., (2006) noted that data of total bacteria counts showed significantly ($P<0.05$) higher values for rabbit for diets contained rice straw treated with fungi than those of control group. Also, they found that cellulolytic bacteria count increased in rabbits fed diet contained 35% rice straw treated with bacteria plus fungi than the groups fed the control diet. Penney et al., (1986) indicated that, there were changes in the digestive tract microflora according to change in pH, diet, and breed.

Fekete (1991) found that, the development and maturity of the gastrointestinal tract development and activity of digestive enzymes in the postnatal period the most important endogenous factors affecting the digestion of nutrients (breed, sex, age, stress and caecotrophy) are outlined. On the other hand, microflora (Bactria, fungi and protozoa) play an important role to help animals to digest the diets that contain non digestible components like roughages, increase the digestibility coefficient by enzymes which produce by this microorganisms and influence the growth and function of the gastrointestinal organs. Meanwhile, Soncini and Cantoni (1978) noted that, bacteria were mainly anaerobic in rabbits *and psedomonas spp* and bacillus strains from caecum synthesized vit B_{12}. In addition to, Forsythe and Parker (1985) found that the dense microbial flora of the ceacum consisted mainly of bacteria (1011 /g) with some yeast cells (106g). Using strictly anaerobic methods, 23% of the direct microscopic cell count was cultivated and 55% of the cultivable bacteria used ammonia as only source of nitrogen and the seureolytic bacteria were *C1*. *Clostridiforme, Bacillus spp and straphlpcpccus spp*. Also, (Parisini et al., 1981) found that, the activity of bacterial flora in the caceum and colon of rabbits was higher with the diets contained free amino acids and oligopeptids 58% than control diets.

Microbial activity evaluation

Data that explained the evaluation of growth microbial strain in drinking water fed to the experimental group rabbits are shown in Table (7).

The results cleared that with increasing the level of adding LOG (from 0 to 30 ml/ liter drinking water) noticed that total aerobic bacterial counts (TABC), total Coliform counts (TCC), feacal coliform counts (FCC), total fungi counts (TFC) and Total entero bacteriacea counts (TEBC) was decreased. Meanwhile, total yeast count (TYC) was increased. All samples of drinking water having total aerobic counts (TABC) less than the recommended safety limit of 10^{-4} cfu/g accirding to the International Dietetics of Association of European Community (IDAEC) and the Egyptian Organization for Standardization.

These results in agreement with those found by(Badr et al., 2013) who pointed that the mean aerobicplate count of water was ($105x10^{-3}$) before addition, while it was ($24x10^{-5}$, $6x10^{-5}$ and $16x10^{-4}$) with adding 1, 2 and 3% of the natural mixture juice (NMJ), respectively. Also, they noted that the growth of the bacterial strains in different supplemented water was less or not detected after the inhibition effect occured resulting to adding the NMJ at various concentrations. Also, they found that the three levels of NMJ inhibited the bacterial growth and total coliform; total feacal coliform counts; total enterobacteriacea counts and *E.coli*. Also, the present results were in harmony with those obtained by (Omer et al., 2015) who noted that increasing addition level of bioactive natural mixture (LOG) realized decreasing in detemination values of TBC, TCC, FCC and TFC. Meanwhile, TYC was increased with increasing the additional level of LOG (5, 10, 15 and 20 ml LOG / kg feed) comparing to control ration that not contained LOG. They also noted that the mean values of TBC, TCC, FCC, TFC, and TYC were ($90x10^{-4}$, $80x10^{-4}$, $70x10^{-4}$, 50×10^{-3} and $15x10^{-2}$, respectively) for control ration (zero LOG). Meanwhile, the corresponding values for the same parameter were ($80x10^{-4}$, $60x10^{-4}$, $45x10^{-4}$, $12x10^{-3}$ and $10x10^{-2}$, respectively) for drinking water before addition of the natural additive. Also, the present results are in agreement with those noted by Aiad et al., (2008) with suckling buffalo calves diets and Ahmed et al., (2009) with growing buffalo calves. In addition to Abdou et al., (1972) noticed that inclusion 5-10 % fresh garlic was sufficient to inhibit the growth of E. coli. On the other hand, our results in agreement with Gherbawy, (1989); (Shiva Kumar, et al., 2010) who showed that the supplementation garlic in broiler diets as active growth promoter was able to reduce the *E. Coli* counts in the intestine when in comparison to the negative control and it improves their growth performance.

CONCLUSION

From the results obtained and under condition that carried out of this work, it can be mentioned that incorporation nature bioactive mixture compose of lemon, onion and garlic (LOG) juice in drinking water of Rex rabbits breed caused an improving in their average daily gain and feed conversion; decreased blood total cholesterol and blood triglyceride with no adverse effect on carcass characteristics. In addition to microbial activity was also improved. The best levels of adding from LOG was 20 ml LOG/ liter dinking water.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

Our team work sent their deep thanks for our freides in both Animal Production Department, National Research Centre, Dokki, Giza, Egypt and Regional Center for Food and Feed, Agriculture Research Center, Ministry of Agriculture, Giza, Egypt for their good helping that make this work possible.

AUTHOR CONTRIBUTIONS

Azza M.M. Badr cooporation in designed, performleed the experiments, field work and following the publication of the MS.

Hamed A.A.Omer cooporation in designed and performleed the experiments, alculated the data, statistical analysis, wrote the manuscript, reveision the MS during the steps of publication.

Neamat I. Bassuony cooporation in prepared bioactive mixture and determined microbiological parameters.

All authors read and approved the final version.

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