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Growth Performance, Blood Constituents and Some of Microbiological Parameters of Broiler Chickens Fed Rations Containing Different Levels of Natural Bioactive Mixture

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Abstract: Total number of 208 one-day-old straight run broiler chicks was housed in batteries and were randomly divided into four groups (each of 52 chicks). Each group was distributed into four replicates (each of 13 chicks) and chicks were used in a feeding trial that continuous for 42 days. Natural bioactive mixture composed of juice of lemon, onion and garlic (LOG) at portions (1.00: 1.00: 0.125/ liter clean water), respectively added to broiler rations at different levels zero, 10, 20 and 30 ml of LOG/ kg feed for (G_1, G_2, G_3 and G_4), respectively to investigate its impact on their productive performance, blood constituents and some of microbiological parameters. Rations were formulated to be isonitrogenous, isocaloric and mycotoxins-free as well as free from any medication as growth promoter or antibiotics and meet the nutrient requirements of the broiler chicks during the starter period from 1-14 days of age, grower period from 15-28 days of age and finisher periods from 29-42 days of age. The results showed that feeding broiler chickens on starter ration in significantly ($P>0.05$) increased final weight (FW). Total body weight gain (TBWG) total feed intake (TFI), meanwhile, feed conversion that expressed as (g. intake/ g. gain) was not significantly ($P>0.05$) affected. Also, feeding broiler chickens on grower ration recorded in significantly ($P>0.05$) increasing in FW, TBWG, TFI, meanwhile, feed conversion of G_4 that received 30 ml LOG containing ration recorded insignificantly ($P<0.05$) decreasing comparing to control (G_1), meanwhile it was not differ significantly ($P>0.05$) with the other groups (G_2 and G_3). In addition to, when broiler chickens received the finisher ration the values of FW, TBWG and TFI were in significantly ($P>0.05$) increased by adding LOG at different levels. Meanwhile, feed conversion that expressed as (g. intake/ g. gain) for group that received 30 ml LOG containing ration (G_4) showed insignificantly ($P<0.05$) decreasing in comparison with the other groups (G_1, G_2 and G_3). Dietary treatments had no significantly ($P>0.05$) effect on values of total proteins, albumin, globulins and albumin: globulins ratio and it caused in significantly ($P>0.05$) decreasing in values of total cholesterol, meanwhile broiler chickens that fed 30 ml LOG/ kg feed containing ration (G_4) was significantly ($P<0.05$) decreased value of triglycerides comparing to (G_1). Values of GOT were significantly ($P<0.05$) affected by adding LOG in the rations. Both G_2 and G_3 showed in significantly ($P>0.05$) decreasing in GOT values, meanwhile, inclusion LOG at different levels in significantly ($P>0.05$) depressed their values of GPT compared to control one. Furthermore, values of creatinine in significantly ($P>0.05$) decreased with adding LOG at 10 or 20 ml/ kg feed (G_2 and G_3), meanwhile, adding 30 ml of LOG/ kg feed (G_4) significantly ($P<0.05$) decreased value of creatinine in comparison with the control (G_1). Values of uric acid were in significantly ($P>0.05$) increased with increasing level of adding from LOG compared to the control one (G_1). Increasing level of addition of bioactive natural mixture (LOG) decreased total bacteria count (TPC), total coliform count (TCC), fecal coliform count (FCC), total mould count (TM). Meanwhile, total yeast count (TY) count was increased with increasing additional level of LOG, at different sampling time that evaluated at (zero, 15, 30, 60 and 90 days of supplementation) with the three different rations (started, grower

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and finishing). It can be mentioned that natural bioactive mixture composed of lemon, onion and garlic juice (LOG) can be used safely in broiler chickens rations up to 30 ml LOG/ kg feed with out occurring any adverse effect on broiler chickens performance, blood parameters with improving their values of liver and kidneys function. In addition to, it can be using this bioactive mixture to improve the utilization of broiler chickens rations throughout decreasing different pathogenic microorganisms.

Key words: Bioactive Mixture · Broiler Chickens · Performance · Blood parameters Microbiological Parameters

INTRODUCTION

As noted by Elagib *et al.* [1]; they reported that feed additives have been widely used to increase animal performance and recently it is used in poultry industry to improve growth, feed efficiency and layers performance.

In addition to An *et al.* [2] suggested that the sub-therapeutic uses of antibiotics to enhance growth and prevent the infectious intestinal diseases have led to a problem of drug residues in final animal products and emerge of new antibiotic-resistance bacteria.

Furthermore, Wenk [3] noted that in many countries, the routine use of antibiotics in poultry diets have been banned and thus, some endeavors are made to develop new in-feed antibiotics substitutes for reducing and treating infectious diseases in poultry industry. The herb and botanicals are increasingly being used in animal feeds, in place of antibiotics, as possible alternative means to prevent infectious diseases and modulate the immune responses.

Today, herbs, spices and medicinal plants have received an increasing attention as possible growth promoter's and additives references. There is an evidence suggests that some of these components have different active substances as observed by Al-Kassie and Witwit [4].

Also, herbs, spices and medicinal plants can have many benefits for the health of broilers and function such as antioxidation ability as found by Hui [5], antimicrobial activity as described by Dorman and Deans [6], enhancing digestion by stimulating endogenous enzymes as reported by Brugalli [7], increase production of digestive enzymes and improve utilization of digestive products by enhancing liver function as recorded by Ziarlarimi *et al.* [8].

The positive effects of herbal supplements on production performance have been mentioned by Tekeli *et al.* [9]. On the other hand, previous studies mentioned that garlic can improve productive performance of broiler chicks and it was used for about 50 years as antibiotic, growth promoters, anti-bacterial, anti-inflammatory, antiseptic, anti-parasitic, immunomodulatory and to enhance growth performance in poultry as shown by Rehman and Munir [10].

The lemon containing active anti-oxidant compounds includes (flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins and isocatechins, in addition some compounds found in natural foods such as vitamins C as recorded by [11-12].

Bioactive ingredients of Turkish and Indian lemon were determined by AL-Jabri and Hossain [13] who noted that the essential oil were DL-limonene (78.92%), α-pinene (5.08%), L-α-terpineol (4.61%), β-myrcene (1.75%), β-pinene (1.47%) and β-linalool (0.95%) for Turkish lemon. Meanwhile, in Indian lemon, essential oil was DL-limonene (53.57%), L-α-terpineol (15.15%), β-pinene (7.44%), α-terpinolene (4.33%), terpinen-4-ol (3.55%), cymene (2.88%) and E-citral (2.38%), respectively.

Both Onion "*Allium ceps* L." and garlic "*Allium sativum* L." have a beneficial effect on lowering the level of cholesterol in blood plasma and serum as found by Abdo *et al.* [14]; have protective effect against many diseases. Moreover, both have valuable nutrients such as vitamins, minerals, essential amino acids and essential fatty acids as reported by Kamanna and Chandrasekhara [15]. Moreover, 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 as reported by Melvin *et al.* [16].

In addition to most of the plant parts of onion and garlic containing compounds with proven anti-bacterial, antiviral, anti-parasitic, anti-fungal properties and have anti-hypertensive, hypoglycemic, antithrombotic, antihyperlipidemic, anti-inflammatory and anti-oxidant activity Lampe [17]. Furthermore, onion bulbs have a beneficial influence on growth performance of broiler chickens Goodarzi *et al.* [18]. In addition to, McCartney [19] reported that fresh onion stimulate blood circulation,

improve immune response and have anti-bacterial effects due to its contents of pungent substances. Moreover, Adibmoradi *et al*. [20] noted that garlic possess anti-microbial activity. Garlic is considered as a plant with antibiotic, anti-cancer, anti-oxidant, immunomodulatory; anti-inflammatory, hypoglycemic and cardiovascular protecting effects Reuter *et al.* [21].

So, this study was carried out to investigate the impact of adding natural bioactive mixture composed of lemon, onion and garlic juice (LOG) at different levels in broiler chickens rations on their growth performance, blood constituents and some of microbiological parameters.

MATERIALS AND METHODS

Aims of the Study: This work aimed to established the effect of adding natural bioactive mixture composed of juice of lemon, onion and garlic (LOG) at portions (1.00: 1.00: 0.125/ liter clean water), respectively to broiler rations at different levels on their productive performance, blood constituents and some of microbiological parameters.

Birds, Feeds and Managements: The present work was carried out at Regional Centre for Food and Feed in cooperation work with Animal Production Department, National Research Centre. Total number of 208 one-day-old straight run broiler chicks was housed in batteries and were randomly divided into four groups (each of 52 chicks). Each group was distributed into four replicates (each of 13 chicks) and chicks were used in a feeding trial that continuous for 42 days, feed and water were offered *ad libitum*.

Rations were formulated to be isonitrogenous, isocaloric and mycotoxins-free as well as free from any medication as growth promoter or antibiotics and meet the nutrient requirements of the broiler chicks during the starter, grower and finisher periods according to the National Research Council [22].

The four tested or experimental treatments were classified as follows:

First group (G_1) fed basal diet that not contained any supplementation and assigned as control group (0% LOG). The others three groups fed rations contained 10, 20 and 30 ml of LOG/ kg feed for second 2nd, 3rd and 4th groups (G_2, G_3 and G_4), respectively.

Experimental birds were fed a starter ration from one to 14 days of age, starter ration contained 23.2% CP and 3045 Kcal ME/Kg ration. From 15 to 28 days of age, the birds were switched to grower ration containing 22% CP and 3164 Kcal ME/Kg feed. While, during 29 to 42 d of age, birds were fed finisher ration contained 20% CP and 3220 Kcal ME/Kg feed as illustrated in (Table 1).

The temperature was set at 32°C on the first day, gradually reduced to 24°C by the end of the third week and until the end of experiment. The light was provided 24 hrs daily during the experiment. All birds were vaccinated against different diseases according to the vaccination programs adopted in most Egyptian chicken broiler farmers.

Chicken performance response variables were determined according to North [23]. Weekly individually body weight, weight gain and feed consumption (g/bird/day) were recorded. Also feed conversion expressed as (g feed/g live body weight gain) was calculated.

Blood Parameters: Blood samples were collected by slaughtering five birds from each treatment at the end of the feeding trial. The blood sample was placed in a plain centrifuge tube for serum separation; serum and plasma samples were stored at -20°C until further biochemical analyses.

Microbiological Quality of Supplemented Rations: Microbiological evaluations for tested ration (0, 10, 20 and 30 ml LOG/ kg feed) were determined five times (at zero, 15, 30, 60 and 90 days of adding the LOG on the rations).

Dietary Microbiological Evaluation: Appropriate dilutions prepared from each sample were used for inoculating different nutrient and selective media. The microbial determinations were applied as follows:

Total Aerobic Viable Counts: Aerobic bacterial counts were estimated on glucose yeast extract nutrient agar medium as the method reported by APHA [24] using pouring plate technique. Suitable plates were counted after incubation at 37°C for 48 hours.

Coliform and Feacal Coliform: Coliform and feacal coliform counts were estimated on Macconkey agar as described by APHA [24] 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.

Table 1: Composition and calculated values of tested rations

Ingredients	Starter ration R_1	Growing ration R_2	Finishing ration R_3
Yellow corn (7.1% CP)	55.81	55.50	62.00
Soybean meal (46% CP)	30.00	31.00	24.00
Corn gluten meal (60.8% CP)	7.50	5.00	6.20
Vegetable oil	2.30	4.63	4.07
Di-calcium phosphate	1.68	1.84	1.70
Limestone	1.28	0.69	0.70
Vitamins & Mineral Mixtures*	0.40	0.40	0.40
Sodium chloride	0.40	0.40	0.40
L-lysine-HCl	0.42	0.31	0.33
DL-Methionine	0.13	0.15	0.12
Choline chloride	0.08	0.08	0.08
Calculated values (%)**			
CP	23.20	22.00	20.00
ME (KCal / kg)	30.45	31.64	32.20
Lysine	1.36	1.30	1.13
Methionine	0.53	0.52	0.47
Methionine+Cystine	0.98	0.94	0.85
Calcium	0.96	0.90	0.85
Available phosphorus	0.45	0.48	0.44

*Each kg of Vitamins & Mineral Mixtures contained 12000 I.U Vit. (A); 2000 I.U Vit. (D_3); 10 mg Vit. (E); 2 mg Vit. (K_3); 1 mg Vit. (B_1); 5 mg Vit. (B_2); 1.5 mgVit. (B_6); 10 μg Vit. (B_{12}); 50g Biotin; 10 mg Pantothenic acid; 30 mg Niacin; 1 mg Folic acid; 60 mg Manganese; 50 mg Zinc; 30 mg Iron; 10 mg Copper; 1 mg Iodine; 0.1 mg Selenium and 0.1 mg Cobalt.

* Values (%) were Calculated according to chemical composition of poultry feed stuffs according to NRC [22].

ME: Metabolizable energy

Yeast Count: Total counts of yeast were determined on Ruse-bengl chloramphenicol agar according to the methods described in Oxpoid Manual [25]. Plates were incubated at 22-25°C for 7 days.

Detection of Salmonella: The methods of Georgala and Boothroyd [26] and Khan and McCaskey [27] was applied by adding 225 ml peptone water as pre enrichment medium to twenty-five g. of each sample and incubated at 37°C for 24 hours. After incubation the culture was streaked on difco brilliant green agar plates and examined after 25-28 hours (on this medium presumptive salmonella appears as pink colonies surrounded by bright red medium).

Enumeration of *Escherichia coli* 0157:H7: Culture media and imurogenetic separation reagents, the enrichment medium was modified tryptone soya broth (mTSB=N) containing novobiocin solution 20 mg/liter of (mTSB) as described by Bolton *et al.* [28] and ISO 6887 [29] and the subculture on medium sorbitol macconkey agar as noted by Difco [30] containing defixime 1ml /liter and potassium telluride 1ml/ liter of sorbitol Macconkey agar [Cefixime etllurite sorbitol Macconkey agar] (CT-SMAC) as described by Zadik *et al.* [31].

Analytical Procedures: Samples of blood serum were used to evaluate the biochemical analysis that includes total proteins according to Henary *et al.* [32], albumin according to Doumas *et al.* [33], total cholesterol according to Allain *et al.* [34], triglycerides according to Fossati and Prencipe [35] activities of amino transferases of GOT and GPT according to Reitman and Frankel [36], uric acid according to Patton and Crouch [37] and creatinine according to Husdan [38]. Serum globulins were determined by subtracting the value of serum albumin from the value of serum total proteins, also A: G ratio was calculated. Commercial diagnostic kits from Biomerieux, France and Quimica Clinica Aplicada (QCA), Amposta, Spain, were used for assay of serum biochemical parameters.

Statistical Analysis: Data collected includes (feed intake, live body weight, feed conversion, blood and microbiological parameters were subjected to statistical analysis as one way analysis of variance according to SPSS [398]. Duncan's Multiple Range Test Duncan [40] was used to separate means when the dietary treatment effect was significant according to the following model:

$$Y_{ij} = \mu + T_i + e_{ij}$$

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

T_i = effect of tested rations levels for i = 1-4, 1 = (basal diet not contained LOG), 2 = basal diet plus 10 ml LOG/ kg feed, 3 = basal diet plus 20 ml LOG/ kg feed and 4 = basal diet plus 30 ml LOG/ kg feed.

e_{ij} = the experimental error.

RESULTS AND DISCUSSION

Broiler Chickens Performance: Data illustrated in Table (2) mentioned that feeding broiler chickens on starter ration during (0-14) days of age in significantly (P>0.05) increased final weight (FW). Total body weight gain (TBWG) total feed intake (TFI), meanwhile, feed conversion that expressed as (g. intake/ g. gain) was not significantly (P>0.05) affected.

Also, feeding broiler chickens on grower ration during (15-28) days of age noticed in significantly (P>0.05) increasing in FW, TBWG, TFI, however, feed conversion that expressed as (g. intake/ g. gain) for group that received 30 ml LOG containing ration (G_4) recorded insignificantly (P<0.05) decreasing comparing to control (G_1), meanwhile it was not differ significantly (P>0.05) with the other groups (G_2 and G_3).

In addition to, when broiler chickens received the finisher ration during (29-42) days of age the values of FW, TBWG and TFI were in significantly (P>0.05) increased by adding LOG at different levels. Meanwhile, feed conversion that expressed as (g. intake/ g. gain) for group that received 30 ml LOG containing ration (G_4) showed insignificantly (P<0.05) decreasing in comparison with the other groups (G_1, G_2 and G_3). These results seemed to be in harmony with those found by Issa and Abo Omar [41] who reported that rations supplemented by garlic did not significantly (P>0.05) affected on the performance of broiler. Also, An *et al.* [2] noted that when white mini broilers chickens fed rations contained 0.3% or 0.5% onion extract had no significant effect on final body weight and weight gain. Meanwhile, Goodarzi *et al.* [18] showed that dietary supplementation of fresh onions bulb at 30 g/ kg diet in Ross 308 broiler chick realized a significantly (P<0.05) increasing in final body weight of broilers at 42nd days of age comparing to the other treatments (0 or 10 g onions/ kg diet). On the other hand, Elagib *et al.* [1] reported that feeding Cobb broiler chicks diets contained 3% garlic powder significantly (P<0.05) increased their feed intake, body weight gain and achieved the best efficiency of feed utilization.

Also, El-Tazi *et al.* [42] recorded that Hubbard broiler chicks fed 3% garlic powder had significantly (P<0.05) heaviest body weight gain, highest feed intake and best feed conversion ratio compared to the others (0, 2 and 4% garlic). Also, Al-Ramamneh *et al.* [43] observed an improvement in body weight when 5% of onion powder was added to broiler rations. It has been reported that onion stimulates the digestion and reducing food transit time in the gastrointestinal tract as noted by Platel and Srinivasan [44]. Also, [45-50] reported that broiler chickens received rations contained garlic powder had the better feed conversion; significantly improved their nutritional performance and that may be due to allicin active ingredients in garlic which promotes the performance of intestinal flora, thereby improving digestion and enhancing the utilization of energy. The biological effects of additional constituents of garlic and onion, such as lectins (the most abundant proteins in garlic and onion), cysteine and methionine (an abundant amino acids), improve the growth of the chicks as found by Corzo-Martinez *et al.* [51]. Also, the present results in agreement with those obtained by Omer *et al.* [52] who studied the impact of adding LOG at (0, 5, 10, 15 and 20 ml/ kg feed) in growing rabbit rations, they reported that dietary treatment had no significant effect on DM intake because the DM intake among the different groups were in the same trend that ranged from 106 to 112 g/h/day in growing rabbits, they concluded that the results might indicate that adding bioactive natural mixture (LOG) to rabbit rations at the experimental levels used had no adverse effect on palatability. Also, the present results in harmony agreement with those found by [53-57]. Furthermore, Ahmed *et al.* [54] reported that average daily gain was increased by 4.8% in growing buffalo calves fed diet contained 2.5% the same bioactive mixture (LOG) that used in the present study. Meanwhile, the same authors noted that average daily gain was significantly (P<0.05) decreased with the higher levels of natural additive (LOG) 5 and 7.5 % compared to 2.5% LOG and insignificantly comparing with the control group calves. Also, the present results in agreement with those noted by Zaki *et al*. [55]; El-Ashry *et al*. [57]; El-Ashry *et al*. [53] and Aiad *et al.* [58]. Also, Hassan and Abdel-Raheem [59] recorded that dry matter intake, final weight; weight gain and feed conversion were slightly improved in calves fed garlic as natural feed additive.

Blood Parameters of the Experimental Groups: Data presented in Table (3) cleared that Dietary treatments had no significantly (P>0.05) effect on values of total proteins, albumin, globulins and albumin: globulins ratio. It in significantly (P>0.05) decreased values of total cholesterol, meanwhile broiler chickens that fed 30 ml LOG/ kg feed (G_4) was significantly (P<0.05) decreased value of triglycerides comparing to that fed ration containing zero ml LOG/ kg feed (G_1), meanwhile G_2 and G_3 that fed 10 or 20 ml LOG/ kg feed were in significantly (P>0.05) decreased their values of triglycerides in comparison with the control (G_1). Values of GOT were significantly (P<0.05) affected by adding LOG in broiler chickens rations, G_4 recorded significantly (P<0.05)

Table 2: Growth performance of experimental groups

Item		Experimental groups G[1]	G[2]	G[3]	G[4]	SEM
Level of LOG adding (ml LOG / kg feed)		0	10 ml	20 ml	30 ml	---
Number of chickens used in the feeding trial		52	52	52	52	---
Starter period (0-14 days)	IW, g	549	544	549	545	1.34
	FW, g	4087	4171	4075	4195	60.88
	TBWG, g	3538	3627	3526	3650	61.37
	Total feed intake, g	4729	4766	4744	4960	70.17
	Feed conversion (g. intake/ g. gain)	1.34	1.31	1.35	1.36	0.16
Grower period (15-28 days)	IW, g	4087	4171	4075	4195	60.88
	FW, g	14561	14895	14585	14625	145.7
	TBWG, g	10474	10724	10510	10430	599.9
	Total feed intake, g	16329	16793	16458	16775	176.3
	Feed conversion (g. intake/ g. gain)	1.56a	1.57ab	1.57ab	1.61b	0.008
Finisher period (29-42 days)	IW, g	14561	14895	14585	14625	145.7
	FW, g	24118	24621	24593	23582	275.3
	TBWG, g	9557	9726	10008	8957	194.7
	Total feed intake, g	17638	17997	17887	18259	204.6
	Feed conversion (g. intake/ g. gain)	1.85 a	1.85 a	1.79 a	2.04 b	0.035

Table 3: Blood parameters of the experimental groups

Item	Experimental groups G_1	G_2	G_3	G_4	SEM
Level of LOG adding (ml LOG / kg feed)	0	10 ml	20 ml	30 ml	---
Number of Samples	5	5	5	5	---
Total proteins (g/dl)	3.15	3.42	3.35	3.30	0.082
Albumin (g/dl)	1.30	1.43	1.40b	1.35	0.036
Globulins (g/dl)	1.85	1.99	1.95	1.95	0.057
Albumin/ globulins ratio	0.70	0.72	0.72	0.69	0.019
Lipid fractions					
Total cholesterol (mg/dl)	120.6	118.5	116.0	114.0	3.13
Triglycerides (mg/dl)	56.63[a]	54.48[a]	48.60[ab]	42.80[b]	3.47
Liver function					
Glutamic oxaloacetic transaminase, GOT (U/l)	87.25[a]	85.75[a]	80.50[ab]	71.50[b]	3.36
Glutamic pyruvic transaminase, GPT (U/l)	20.25	19.50	18.75	17.00	0.92
Kidneys function					
Creatinine (mg/dl)	0.23[a]	0.20[ab]	0.19[ab]	0.18[b]	0.008
Uric acid (mg/dl)	2.29	2.47	2.69	2.75	0.17

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

LOG: Natural bioactive mixture composed of lemon, onion and garlic juice at portions (0.125: 1.00: 1.00 / liter clean water

decreasing in GOT value comparing the G_1 (control), however, both G_2 and G_3 in significantly (P>0.05) decreased value of GOT compared to G_1. On the other hand, inclusion LOG at different level (from 10 to 30 ml LOG/ kg feed) in significantly (P>0.05) depressed their values of GPT comparing to control (G_1). Moreover, values of creatinine in significantly (P>0.05) decreased with adding LOG at 10 or 20 ml/ kg feed, meanwhile, adding 30 ml of LOG/ kg feed (G_4) significantly (P<0.05) decreased value of creatinine in comparison with the control (G_1). Values of uric acid were in significantly (P>0.05) increased with increasing level of adding from LOG compared to the control one (G_1). Data concerning of biochemical and hematological blood parameters mentioned that garlic and onion contain organic sulfur compounds including S-methylcysteine sulfoxide and S-allylcysteine sulfoxide with antioxidant and antiperoxide activity as showed by Corzo-Martinez *et al.* [51]. These compounds are related to decrease the blood lipid, liver protein and glucose [60, 61]. Goodarzi *et al*. [18] noted that using onion bulbs in the broiler diet can depressed their triglycerides and total cholesterol values.

Table 4: Effect of dietary treatment time on some parameter of microbial count for starter ration

Item		Microbiological parameters								
Time	Treatment	T.P.C	T.C.C	F.C.C	T.M	T.Y	*E. coli*	*S. aureus*	*S. spp.*	*C. jejuni*
Zero	Control	70X 10^6	50X 10^6	30X 10^5	3X 10^5	6X 10^3	+	+	-	+
	1% LOG	50X 10^6	30X 10^6	20X 10^4	1X 10^4	4X 10^3	+	+	-	+
	2% LOG	32X 10^4	25X 10^4	19X 10^4	16X 10^2	15X10^3	-	-	-	-
	3% LOG	25X 10^3	12X 10^3	9X 10^3	9X 10^2	20X 10^3	-	-	-	-
15 days	Control	90X 10^7	54X 10^6	30X 10^6	3X 10^5	5X 10^2	+	+	-	+
	1% LOG	60X 10^6	40X 10^6	32X 10^6	2X 10^4	5X 10^2	+	+	-	+
	2% LOG	52X 10^4	30X 10^4	27X 10^4	2X 10^2	16X10^2	-	-	-	-
	3% LOG	30X 10^3	25X 10^3	15X 10^3	8X 10^2	22X 10^2	-	-	-	-
30 days	Control	12X 10^8	16X 10^7	38X 10^6	6X 10^5	5X 10^2	+	+	-	+
	1% LOG	40X 10^4	16X 10^3	9X 10^3	1X 10^4	12X 10^3	+	+	-	+
	2% LOG	20X 10^4	49X 10^3	25X 10^4	3X 10^3	16X10^3	-	-	-	-
	3% LOG	24X 10^3	35X 10^3	23X 10^3	2X 10^2	22X 10^3	-	-	-	-
60 days	Control	64X 10^8	26X 10^7	20X 10^7	10X 10^6	2X 10^2	+	+	-	+
	1% LOG	55X 10^3	12X 10^3	7X 10^3	2X 10^3	20X 10^2	+	+	-	+
	2% LOG	42X 10^3	36X 10^3	27X 10^3	1X 10^3	19X10^3	-	-	-	-
	3% LOG	37X 10^3	32X 10^3	13X 10^3	2X 10^2	31X 10^3	-	-	-	-
90 days	Control	10X 10^9	30X 10^7	25X 10^7	10X 10^6	----	+	+	-	+
	1% LOG	50X 10^3	10X 10^6	22X 10^2	2X 10^2	30X 10^2	+	+	-	+
	2% LOG	35X 10^3	29X 10^2	21X 10^2	1X 10^2	22X10^3	-	-	-	-
	3% LOG	30X 10^3	23X 10^2	17X 10^2	1X 10	35X 10^3	-	-	-	-

T.P.C: Total bacterial count. T.C.C: Total coliform count. F.C.C: Faecal coliform count.

T.M: Total mold count. T.Y: Total yeast count E. coli: E. coli detected.

S. aureus: Staphylococcus detected. S. ssp: Salmonella spp. Detected. C. jejuni: campylobacter jejuni detected.

LOG: Natural bioactive mixture composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ liter clean water

Allicin and its derivative compounds are the primary active substances responsible for the hypolipidemic and hypocholesterolemic effects of onion and garlic [51, 62]. These compounds possibly impact hypercholesterolemia by inhibiting hepatic cholesterol biosynthesis, enhancing cholesterol turnover to bile acids as noted by Srinivasan and Sambaiah [63] or inhibit their cholesterol absorption from the intestinal lumen as reported by Slowing *et al.* [64]. On the other hand, An *et al*. [2] fed White mini broilers diets contained 0, 0.3 or 0.5% of onion extract, they not found any significant differences in the activities of GOT and GPT in blood serum among different groups. Meanwhile, El-Demerdash *et al*. [65] showed that incorporation onion juice enhanced levels of GOT and GPT could restore to normal levels in alloxan diabetic rats. Also, they mentioned that onion juice exerted antioxidant effects and alleviated the tissue damage caused by alloxan-induced diabetes. A significant positive effect on antioxidant activity was noticed with onion extract as noted by Chang *et al.* [66]. The measurement of serum GOT and GPT activities indicative of tissue damage in bird is also a valuable tool to evaluate a safe inclusion level for non-conventional feedstuff and new additives as reported by Diaz *et al.* [67]. Furthermore, An *et al*. [2] recorded that levels of serum cholesterol ester and phospholipid were not affected by adding 0.3 or 0.5% of onion in White mini broilers diets. In addition to, they watched that the concentrations of serum free cholesterol and triacylglycerol were significantly ($P<0.01$) decreased in groups fed diets with onion extract relative to control.

Microbiological Quality of Supplemented Rations: Microbiological quality of LOG supplementation in broiler chickens rations at different levels (10, 20 and 30 ml/ kg feed) that illustrated in Tables (4, 5, 6, 7 and 8) showed that increasing level of addition of bioactive natural mixture (LOG) decreased total bacteria count (TPC), total coliform count (TCC), fecal coliform count (FCC), total mould count (TM). Meanwhile, total yeast count (TY) count was increased with increasing additional level of LOG, at different sampling time that evaluated at (zero, 15, 30, 60 and 90 days of supplementation) with the three different rations (started, grower and finishing). The present results in harmony with those obtained by Omer *et al.* [52] who used LOG in rabbit rations and drinking water at 5, 10, 15 and 20 ml/ kg feed and they noted that increasing level of addition of bioactive natural mixture (LOG) decreased TPC, TCC, FCC and TM.

Table 5: Effect of dietary treatment time on some parameter of microbial count for grower ration

Item		Microbiological parameters								
Time	Treatment	T.P.C	T.C.C	F.C.C	T.M	T.Y	*E. coli*	*S. aureus*	*S. spp.*	*C. jejuni*
Zero	Control	45X 10^7	39X 10^7	25X 10^6	3X 10^5	3X 10	+	+	-	+
	1% LOG	40X 10^6	35X 10^6	20X 10^5	2X 10^4	5X 10	+	+	-	+
	2% LOG	3X 10^5	70X 10^4	50X 10^4	4X 10^2	10X10	-	-	-	-
	3% LOG	3X 10^4	32X 10^3	27X 10^3	14X10	17X 10	-	-	-	-
15 days	Control	60X 10^7	55X 10^5	33X 10^5	17X 10^4	25X 10^2	+	+	-	+
	1% LOG	50X 10^6	41X 10^5	35X 10^4	12X 10^4	30X 10^2	+	-	-	+
	2% LOG	25X 10^5	17X 10^4	10X 10^4	7X 10^3	35X10^2	-	-	-	-
	3% LOG	22X 10^4	20X 10^3	8X 10^3	2X 10^3	40X 10^2	-	-	-	-
30 days	Control	80X 10^7	40X 10^6	20X 10^5	33X 10^4	15X 10^2	+	+	-	+
	1% LOG	50X 10^4	31X 10^4	15X 10^3	1X 10^4	24X 10^2	+	+	-	+
	2% LOG	40X 10^3	20X 10^3	13X 10^2	2X 10^3	30X10^3	-	-	-	-
	3% LOG	30X 10^3	15X 10^3	9X 10^2	7X 10	35X 10^3	-	-	-	-
60 days	Control	14X 10^8	90X 10^6	60X 10^6	10X 10^5	12X 10^2	+	+	-	+
	1% LOG	42X 10^4	39X 10^3	30X 10^2	3X 10^3	30X 10^2	-	-	-	+
	2% LOG	36X 10^3	27X 10^3	25X 10^2	1X 10^3	32X10^2	-	-	-	-
	3% LOG	24X 10^3	20X 10^3	10X 10^2	2X 10	40X 10^3	-	-	-	-
90 days	Control	16X 10^8	12X 10^7	90X 10^6	12X 10^5	3X 10	+	+	-	+
	1% LOG	40X 10^3	35X 10^3	26X 10^2	2X 10^2	30X 10^2	-	-	-	-
	2% LOG	31X 10^3	20X 10^3	6X 10^2	1X 10^2	34X10^2	-	-	-	-
	3% LOG	21X 10^3	14X 10^2	3X 10	2X 10	42X 10^3	-	-	-	-

T.P.C: Total bacterial count. T.C.C: Total coliform count. F.C.C: Faecal coliform count.

T.M: Total mold count. T.Y: Total yeast count E. coli: E. coli detected.

S. aureus: Staphylococcus detected. S. ssp: Salmonella spp. Detected. C. jejuni: campylobacter jejuni detected.

LOG: Natural bioactive mixture composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ liter clean water.

Table 6: Effect of dietary treatment time on some parameter of microbial count for finisher ration

Item		Microbiological parameters								
Time	Treatment	T.P.C	T.C.C	F.C.C	T.M	T.Y	*E. coli*	*S. aureus*	*S. spp.*	*C. jejuni*
Zero	Control	80X 10^7	50X 10^7	40X 10^6	9X 10^5	1X 10^2	+	+	-	+
	1% LOG	70X 10^6	60X 10^6	35X 10^5	3X 10^4	9X 10^2	+	+	-	+
	2% LOG	30X 10^4	19X 10^4	17X 10^3	2X 10^2	2X10^3	-	-	-	-
	3% LOG	15X 10^4	10X 10^4	13X 10^3	8X10	2X 10^4	-	-	-	-
15 days	Control	96X 10^7	70X 10^{6c}	55X 10^6	10X 10^5	20X 10^2	+	+	-	+
	1% LOG	70X 10^7	50X 10^6	42X 10^6	5X 10^4	30X 10^2	+	-	-	+
	2% LOG	35X 10^4	31X 10^4	34X 10^3	3X 10^3	34X10^2	-	-	-	-
	3% LOG	23X 10^4	19X 10^4	26X 10^3	1X 10^3	34X 10^2	-	-	-	-
30 days	Control	50X 10^8	80X 10^6	65X 10^6	35X 10^5	16X 10^2	+	+	-	+
	1% LOG	17X 10^4	60X 10^3	17X 10^2	2X 10^4	20X 10^2	+	+	-	+
	2% LOG	13X 10^4	15X 10^3	12X 10^2	1X 10^2	33X10^3	-	-	-	-
	3% LOG	10X 10^4	10X 10^3	9X 10^2	3X 10	40X 10^3	-	-	-	-
60 days	Control	12X 10^8	90X 10^7	75X 10^7	44X 10^5	2X 10	+	+	-	+
	1% LOG	22X 10^3	18X 10^3	12X 10^2	3X 10^3	22X 10^2	-	-	-	-
	2% LOG	25X 10^3	12X 10^3	9X 10^2	1X 10^2	35X 10^3	-	-	-	-
	3% LOG	20X 10^3	9X 10^3	7X 10^2	2X 10	43X 10^3	-	-	-	-
90 days	Control	14X 10^8	10X 10^8	90X 10^7	41X 10^5	----	+	+	-	+
	1% LOG	25X 10^3	15X 10^3	10X 10^2	2X 10^3	13X 10^2	-	-	-	-
	2% LOG	23X 10^3	10X 10^3	6X 10^2	1X 10^2	35X10^2	-	-	-	-
	3% LOG	19X 10^3	5X 10^3	3X 10	2X 10	54X 10^3	-	-	-	-

T.P.C: Total bacterial count. T.C.C: Total coliform count. F.C.C: Faecal coliform count.

T.M: Total mold count. T.Y: Total yeast count E. coli: E. coli detected.

S. aureus: Staphylococcus detected. S. ssp: Salmonella spp. Detected. C. jejuni: campylobacter jejuni detected.

Table 7: Isolated fungi from different rations at 1st day before treatment

Item Rations	Treatments	Isolated fungi at 1st day before treatments				
Started ration	Control	*Aspergillus niger*	*Fusarium spp.*	*Penicillum spp.*	*Aspergillus spp.*	
	1% LOG	*Aspergillus niger*.		*Alternaria spp.*		
	2% LOG	*Penicillum digtatum*.		*Aspergillus spp.*		
	3% LOG	*Alternaria spp.*		*Penicillum spp.*		
Grower ration	Control	*Aspergillus flavus*		*Aspergillus spp.*		
	1% LOG	*Aspergillus niger*	*Aspergillus flavus*	*Fusarium spp.*	*Aspergillus spp.*	
	2% LOG	*Baetena*	*Penicillum spp.*		*Aspergillus spp.*	
	3% LOG	…….…….				
Finisher ration	Control	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus fumingatus*	*Fusarium spp*	*Penicillum spp.*
	1% LOG	*Aspergillus niger*	*Fusarium spp*	*Penicillum spp*	*Aspergillus fumingatus*	
	2% LOG			*Aspergillus niger*		
	3% LOG	*Aspergillus niger*	*Penicillum digitatum*		*Aspergillus flavus*	

All sample's analysis are free toxins from zero time.

LOG: Natural bioactive mixture composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ liter clean water.

Table 8: Isolated fungi from different rations at 90 days of treatment

Item Rations	Treatments	Isolated fungi after 90 days of treatments			
Started ration	Control	*Fusarium spp.*	*Penicillum spp.*	*Aspergillus spp.*	
	1% LOG	*Aspergillus niger*		*Penicillum spp*	
	2% LOG	*Aspergillus nidulans*		*Aspergillus spp.*	
	3% LOG	*Aspergillus cadidus*		*Aspergillus nidulans*	
Grower ration	Control	*Fusarium spp.*	*Aspergillus niger*	*Aspergillus spp.*	
	1% LOG	*Aspergillus niger*	*Aspergillus cadidus*	*Aspergillus nidulans*	
	2% LOG	*Penicillum digitatum*		*Penicillum funiculosum*	
	3% LOG		Contaminated		
Finisher ration	Control	*Aspergillus niger*	*Aspergillus fumingatus*	*Penicillum spp*	*Aspergillus parasiticus*
	1% LOG	*Aspergillus spp.*	*Aspergillus flavus*		*Fusarium spp.*
	2% LOG		*Aspergillus niger*		
	3% LOG	*Aspergillus niger*			*Penicillum digitatum*

All sample's analysis are free toxins from zero time.

LOG: Natural bioactive mixture composed of lemon, onion and garlic juice at portions (1.00: 1.00: 0.125/ liter clean water

Meanwhile, TY count was increased with increasing additional level of LOG. The mean of total bacteria count (TPC), total coliform count (TCC), fecal coliform count (FCC), total mould count (TM) and total yeast count (TY) in experimental rations and drinking water with or without the bioactive natural mixture (LOG) additive. The mean values of TPC, TCC, FCC, TM and TY were (90×10^{-4}, 80×10^{-4}, 70×10^{-4}, 50×10^{-3} and 15×10^{-2}, respectively) for control ration (R_1). Meanwhile, the corresponding values for the same parameter were (80×10^{-4}, 60×10^{-4}, 45×10^{-4}, 12×10^{-3} and 10×10^{-2}, respectively) for drinking water before addition of the natural additive. Total aerobic counts were not higher than the recommended safety. For instance, all samples having total aerobic counts (TPC) less than the recommended safety limit of 10^{-4} cfu/g proposed by the International Dietetics of Association of European Community (IDAEC) and the Egyptian standards. Also, our results in agreement with those reported by Aiad *et al.* [58] and Ahmed *et al.* [54]. The growth of the bacterial strains in different addition rations was less or not detected after the inhibition effect of the juice at various concentrations. The four levels of juice inhibited the *Salmonella spp*. in rations and water intake Omer *et al.* [52].

Natural bioactive mixture (LOG) contains garlic, onion and lemon juice, garlic contains 0.3-0.5 *allicin* and antimicrobial component as noted by Shelef [68]. According to Kumar and Berwal [69] and Zaika and Kissinger [70] the gram-positive are generally more sensitive to *allicin* than gram-negative bacteria. Acetic acid bacteria are the most resistant among the gram positive bacteria. Abdou *et al*. [71] indicated that 5-10 % fresh garlic was sufficient to inhibit the growth of *E. coli*. The same trend was observed in onion.

The microorganisms employing new mechanisms to survive Sekyere and Asante [72]. From the earlier studies, it is obvious that most of the phytochemicals differ significantly in their structures and properties. These differences may be attributed to the differences in the cell wall constituents of bacteria which vary among the gram-positive and gram-negative bacteria as noted by Yao and Moellering [73]; Siddiqui and Begum (74].

CONCLUSION

It can be mentioned that, under conditions similar to those available during carrying of this study, natural bioactive mixture composed of lemon, onion and garlic juice (LOG) can be used safely in broiler chickens rations up to 30 ml LOG/ kg feed with out occurring any adverse effect on broiler chickens performance, blood parameters with improving their values of liver and kidneys function. Also, it can be using this bioactive mixture to improve the utilization of broiler chickens rations throughout decreasing different pathogenic microorganisms.

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