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Productive Performance, Carcass Characteristics, Amino Acids, Fatty Acids Profile and Blood Parameters of Broiler Chicken Fed Rations Containing Different Levels of Natural Bioactive Mixture

[1]Hamed A.A. Omer, [2]Hady F.A. Motawee, [2]Neamat I. Bassuony and [2]Shereen Nada

[1]Animal Production Department, National Research Centre, 33 El-Bohouth Street,
P.O. Box: 12622, Dokki, Giza, Egypt
[2]Regional Center for Food and Feed, Agriculture Research Centre,
Ministry of Agriculture, Giza, Egypt

Abstract: This study aimed to evaluate the impact of adding different levels of natural bioactive mixture composed of (Lemon, Onion and Garlic juice) in broiler chickens rations. Total number of 208 one-day-old straight run broiler chicks were housed in batteries and were randomly divided into four groups (each of 52 chicks). Each group divided into four replicates (each of 13 chicks) and chicks raised for 42 days, feed and water were provided *ad libitum*. The experimental rations were classified as the following: 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, third and fourth groups (G_2, G_3 and G_4), respectively. All birds were fed a starter ration from one to 14 days of age containing 23.2% CP and 3045 Kcal ME/Kg diet. 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, they were fed finisher ration containing 20% CP and 3220 Kcal ME/Kg feed. The results showed that incorporation LOG had no significant ($P>0.05$) effect on final weight, total body weight gain and total feed intake during the three different stages of feeding. Chickens received 10 ml LOG/ kg feed recorded the best feed conversion (1.31) during starter period, meanwhile, that received 30 ml LOG/ kg feed realized the lowest feed conversion (1.61) during the grower period. Furthermore, chickens received 20 ml LOG/ kg feed recorded the best feed conversion (1.79 and 1.63) throughout the both finisher and over the entire period, respectively. Dietary treatments had no significant effect ($P>0.0\%$) on dressing percentages, liver, gizzard and abdominal fat that evaluated as weight per grams or that expressed as % of carcass weight. However, heart weight or heart as % of carcass weight was significantly ($P<0.05$) decreased. Inclusion LOG at different levels had no significant effect on moisture and ash contents of carcass meat comparing to the control, meanwhile, it significantly ($P<0.05$) increased their values of crude protein, but, it significantly ($P<0.05$) decreased their content of fat in carcass meat. Except for the isoleucine the others essential amino acid profile was affected by inclusion LOG in chicks rations. Meanwhile, except for the aspartic the other non-essential amino acid profile was affected by inclusion LOG in broiler chickens rations. Incorporation LOG had no significant effect ($P>0.05$) on all fatty acids profile of carcass meat determined. Mono unsaturated fatty acids (MUFA); Poly unsaturated fatty acids (PUFA) and total unsaturated fatty acids (TUFA) of carcass meat were insignificantly ($P>0.05$) increased. Total protein content insignificantly decreased, meanwhile, albumin and globulins contents were insignificantly increased. Total cholesterol, triglycerides, GOT and GOT values were decreased with increasing the level supplementation of LOG. All hematological blood parameters were not significantly affected. It can be mentioned that adding natural bioactive mixture composed of lemon, onion and garlic juice in the rations of broiler chickens improves their feed efficiency and boy weight without occurred any adverse effect on their carcass characteristics or meat quality with decreasing the blood cholesterol, triglycerides, GOT and GPT.

Key words: Bioactive Mixture; Broiler; Performance; Carcass; Meat quality; Blood

Corresponding Author: Hamed A.A. Omer, Nimal Production Department, National Research Centre,
33 El-Bohouth Street, P.O. Box: 12622, Dokki, Giza, Egypt.

INTRODUCTION

For many years 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 [1-3].

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 [4, 5]. 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 [6].

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 [7]. Also, they can have many benefits for the health of broilers and function such as antioxidation ability [8], antimicrobial activity [9], enhancing digestion by stimulating endogenous enzymes [10], increase production of digestive enzymes and improve utilization of digestive products by enhancing liver function [11].

The active anti-oxidant compounds in lemon are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins and isocatechins, also some compounds found in natural foods such as vitamins C [12-14]. Also, Lemon peel contains phenolic compounds, such as flavonoids (flavanones, flavonols, flavones), phenolic acids (ferulic, p-coumaric and sinapic acids), as well as vitamin C (ascorbic acid) as described by [15], which have been linked to anti-microbial [16], anti-cancer [17] and anti-oxidant properties [18, 19].

Onion "*Allium ceps* L." and garlic "*Allium sativum* L." are cultivated widely in Egypt and used by Egyptian farmers since long time ago in poultry diets because they believed that, both have a beneficial effect on lowering the level of cholesterol in blood plasma and serum [20]; have protective effect against many diseases. Moreover, both have valuable nutrients such as vitamins, minerals, essential amino acids and essential fatty acids [21]. In addition to, 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 [22, 23].

Most of the plant parts contain compounds with proven anti-bacterial, antiviral, anti-parasitic, anti-fungal properties and have anti-hypertensive, hypoglycemic, antithrombotic, antihyperlipidemic, anti-inflammatory and anti-oxidant activity [24]. Furthermore, many authors found beneficial influence for onion bulbs on growth performance of broiler chickens [5, 25-26]. In addition to, McCartney [27] and Ibrahiem *et al*. [28] 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.* [29] 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 [30].

The positive effects of herbal supplements on production performance and carcass quality have been demonstrated by [31, 32]. 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 [3, 33-36].

So, this work was carried out to establish the influence of adding natural bioactive mixture composed of lemon, onion and garlic) juice (LOG) at different levels on growth performance, carcass characteristics, meat quality that includes: chemical composition, amino acids & fatty acids profiles of meat and blood constituents of broiler chickens.

MATERIALS AND METHODS

Birds, Feeds and Managements: This 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 divided into four replicates (each of 13 chicks) and chicks raised for 42 days, feed and water were provided *ad libitum*.

Table 1: Formula and calculated values (%) of different experimental rations

Ingredients	Starter ration	Grower ration	Finisher ration
Yellow corn	55.81	55.50	62.00
Soybean meal	30.00	31.00	24.00
Corn gluten meal	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
Total	100	100	100
Calculated values (%)*			
Crude protein (CP)	23.20	22.00	20.00
Metabolizable energy (ME), kcal/ kg	3045	3164	3220
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 [37]

This work aimed to investigate the impact 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 performance, carcass characteristics, meat quality includes (amino acids, fatty acids profile and chemical composition of meat) and blood constituents.

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 starter, grower and finisher periods according to the National Research Council [37].

The four experimental treatments were classified as the following:

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, third and fourth groups (G_2, G_3 and G_4), respectively.

All birds were fed a starter ration from one to 14 days of age containing 23.2% CP and 3045 Kcal ME/Kg diet. 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, they were fed finisher ration containing 20% CP and 3220 Kcal ME/Kg feed as described 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 throughout the experiment. All experimented 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 [38]. 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.

Carcass Traits: At the end of the feeding trial six birds were selected randomly from each group, weighed individually after an overnight fasting with only water allowed, then they were slaughtered. After bleeding they were scalded in hot water, hand-plucked and washed and eviscerated.

At the end of feeding trial, five representative birds from each treatment were randomly chosen to determine the carcass parameters according to [39]. Birds were performed according to the Islamic rules. Animals were weighed just before slaughter, slaughter weight (SW) was recorded and as well as after complete bleeding.

Total edible offal's (Giblets) included liver, gizzard, heart and spleen were weighed.

Hot carcass weight (CW1) and hot carcass weight included giblets (CW2) were recorded to calculate dressing percentages as the following:

- Dressing percentages (DP1) = CW_1 / SW
- Dressing percentages (DP2) = CW_2 / SW

Blood Parameters: Two blood samples were collected through out the slaughtering processing from the slaughtered birds (five in each treatment). The first blood sample was collected on EDTA and was used for hematological determination. Meanwhile, the second 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.

Analytical Procedures: Chemical analysis includes moisture; crude protein (CP); ether extract (EE) and ash percentages were determined in broilers meat according to the AOAC [40] method.

Amino acids composition in broilers meat was estimated according to the method described by Millipore Cooperative [41] using HPLC and the modification of PICO-TAG methods.

On the other hand, fatty acid profiles were conducted through out extracted lipids from broiler meat by diethyl ether as described by the AOAC [40]. The extracted lipids were converted to methyl esters as described by AOAC [42] and analyzed for individual fatty acids (C14: 0 to C20: 4) using a gas chromatograph (3400, Varian Inc., Walnut Creek, CA) fitted with a flame ionization detector. Gas chromatography parameters were as follows: the column temperature was 50°C for 3 min and then increased to 220°C at 4°C/min and was held for 15 min. The injector temperature was 200°C and the detector temperature was 250°C. The flow rates of the carrier gases (hydrogen and oxygen) were 30 and 300 ml/min, respectively. Identification and quantification of individual fatty acids was made by using a standard fatty acid methyl ester mixture (2010, Matreya Biochemical LLC, Pleasant Gap, PA).

Hematological parameters of collected blood samples were described by Weiss and Wardrop [43] for the red blood cell count (RBC's) and white blood cell count (WBC's); hemoglobin (Hb) concentration were determined according to [44, 45].

Meanwhile, samples of blood serum were used to evaluate the biochemical analyses that includes total proteins [46], albumin [47], total cholesterol [48], triglycerides [49] activities of aminotransferases of GOT and GPT [50], uric acid according to [51] and creatinine according to [52]. 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 of feed intake, live body weight, feed conversion, carcass, amino acids, fatty acids and blood parameters were subjected to statistical analysis as one way analysis of variance according to SPSS [53]. Duncan's Multiple Range Test Duncan [54] was used to separate means when the dietary treatment effect was significant according to the following model:

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

where: Y_{ij} = observation. μ = overall mean.
T_i = effect of tested diet 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

Experimental Rations: Data illustrated in Table (1) showed that experimental rations were formulated to cover the requirements of broiler chickens at different stages of ages as recommended by NRC [37].

Growth Performance: Data obtained in Table (2) mentioned that incorporation natural bioactive mixture (LOG) in broiler chickens rations had no significant ($P>0.05$) effect on final weight, total body weight gain and total feed intake during the three different stages of feeding {starter period (0-14 days), growing period (15-28 days) and finishing period (29-42 days) and over the entire period from 0-42 days of age}. However, G_2 that received ration contained 10 ml LOG/ kg feed recorded the best feed conversion (1.31) during starter period, while, G_4 that received ration contained 30 ml LOG/ kg feed realized the lowest feed conversion (1.61) during the grower period. Meanwhile, G_3 that fed ration contained 20 ml LOG/ kg feed showed the best values of feed conversion (1.79 and 1.63) throughout the both finisher and over the entire period, respectively. These results seemed to be in harmony with those obtained by Ademola *et al.* [55]; Onibi *et al.* [56]; Ziarlarimi *et al.* [57];

Table 2: Effect of adding different levels of LOG on growth performance of experimental groups

Item		Experimental groups G_1 Basal ration	G_2 10 ml LOG / kg feed	G_3 20 ml LOG / kg feed	G_4 30 ml LOG / kg feed	SEM
Number of chickens		52	52	52	52	---
Starter period (0-14 days)	IW, g	549	544	4766	545	1.34
	FW, g	4087	4171	549	4195	60.88
	TBWG, g	3538	3627	4075	3650	61.37
	Total feed intake, g	4729	3526	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)	1.56[a]	1.57[ab]	1.57[ab]	1.61[b]	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
Over the entire period (0- 42 days)	IW, g	549	544	549	545	1.34
	FW, g	24118	24621	24593	23582	275.3
	TBWG, g	23569	24077	24044	23037	275.6
	Total feed intake, g	38698	39556	39091	39995	387.2
	Feed conversion (g. intake/ g. gain)	1.64[a]	1.64[a]	1.63[a]	1.74[b]	0.013

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

SEM: standard error of the mean.

IW= initial weight FW= Final weight TBWG= Total body weight gain

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

Issa and Abo Omar [58] who reported that rations supplemented by garlic did not significantly (P>0.05) affected on the performance of broiler. In addition to, An *et al.* [5] noticed 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. In contrast, Goodarzi *et al.* [26] observed that dietary supplementation of fresh onions bulb at 30 g/ kg diet in Ross 308 broiler chick caused 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). Also, they noticed that birds fed 30 g onion/ kg diet had the highest feed intake all periods. In addition, Elagib *et al.* [3] 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.* [59] 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.* [60] 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 [61]. Also, Tollba and Hassan [62]; Al-Homidan [63]; Fayed *et al.* [64]; Eid and Iraqi [65]; Oleforuh-Okoleh *et al.* [66]; Al-Ramamneh [67] noticed that fed broiler chickens ration 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 [68].

Carcass Characteristics

Edible Offals and Dressing Percentages: Data of Table (3) cleared that incorporation LOG at different levels of broiler chickens had no significant effect (P<0.0%) on dressing percentages, liver, gizzard and abdominal fat that evaluated as weight per grams or that expressed as % of carcass weight. Meanwhile, heart weight or heart as % of

Table 3: Effect of adding different levels of LOG on carcass characteristics of experimental groups

Item		Experimental groups				SEM
		G_1 Basal ration	G_2 10 ml LOG / kg feed	G_3 20 ml LOG / kg feed	G_4 30 ml LOG / kg feed	
Slaughtering weight (LBW)		1880^{ab}	1817^{bc}	1777^{c}	1883^{a}	14.01
Carcass weight, g (CW_1)		1367^{a}	1340^{ab}	1297^{b}	1393^{a}	12.22
Edible offals (Giblets)						
Liver	Weight, g	39.75	38.54	37.52	41.62	0.99
	% of CW_1	2.91	2.88	2.89	2.99	0.077
Gizzard	Weight, g	37.87	33.98	34.44	35.12	0.92
	% of CW_1	2.77	2.54	2.66	2.52	0.072
Heart	Weight, g	11.29^{a}	8.20^{b}	7.89^{b}	8.81^{b}	0.38
	% of CW_1	0.83^{a}	0.61^{b}	0.61^{b}	0.63^{b}	0.029
Spleen	Weight, g	1.93^{b}	2.90^{a}	1.65^{b}	2.27^{ab}	0.015
	% of CW_1	0.14^{b}	0.22^{a}	0.13^{b}	0.16^{b}	0.011
Total edible offals (Giblets)	Weight, g	90.84^{a}	83.62^{ab}	81.50^{b}	87.82^{ab}	1.37
	% of CW_1	6.65	6.24	6.28	6.30	0.12
Abdominal fat	Weight, g	31.16	26.38	22.50	31.18	2.00
	% of CW_1	2.28	1.97	1.73	2.24	0.15
Carcass weight plus total edible offals (CW_2)		1458^{a}	1424^{ab}	1379^{b}	1481^{a}	12.38
Dressing percentages expressed as:						
CW_1/LBW		72.71	73.75	72.99	73.98	0.57
CW_2/LBW		77.55	78.37	77.60	78.65	0.62

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

SEM: standard error of the mean.

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

Total edible offals (giblets) include liver, gizzard, heart and spleen

carcass weight was significantly (P<0.05) decreased. On the other hand, G_2 that received 10 ml LOG/ kg feed recorded significantly (P<0.05) increasing in spleen weight per gram and spleen as % of carcass weight in comparison with the control one. (2.90g vs. 1.93g) and (0.22% vs. 0.14%) for G_2 and control (G_1), respectively.

The present results of edible offals and dressing percentages in agreement with those found by Aji *et al.* [25] and An *et al.* [5] who noted that the carcass yield and the relative weights of edible parts of broiler were not affected by dietary treatments that contained garlic and onion. Meanwhile, Eltazi [69] noted that addition of different mixture levels of garlic and ginger powder to broiler diets significantly (P<0.05) decreased the percentages of abdominal fat, liver and gizzard in comparison with the control diet. Also, the present results were near from the results that obtained by Al-Ramamneh [67] who noted that supplemented 2.5 kg/ ton of garlic or onion in Ross broiler chicks diet had no significant differences in chest, thigh, back, wings and neck, nevertheless, there was a significant decrease in percentage of abdominal fat weight comparing to control. Also, they observed that liver weight was significantly (P<0.05) increased in treated group in comparison with the control. Also, they reported that the possible combination of garlic and onion has lipotropic effects that affect lipid metabolism through fatty acid transport. This can increase lipid utilization and decrease abdominal fat. Furthermore, Emadi and Kermanshahi [70] reported that supplementation of turmeric as feed additives at (0.75%) in broiler rations leads to improve their carcass quality, lean meat and significant decrease in abdominal fat pad up to 57% level and heart weights to live body weight. Furthermore, it causes higher dressing percent up to 57% level, increased the liver weight, spleen weight and whole giblets weight [71-73]. Moreover, Onibi *et al.* [56] noted that no significant (P>0.05) effect of garlic supplementation on dressing percentage. The relative weights of the abdominal fat were numerically lower for broilers fed supplementary garlic compared with those fed the control diet. This suggests that garlic supplementation could reduce fat deposition [74].

Meat Quality

Chemical Composition of Carcass Meat: Data illustrated in Table (4) showed that incorporation LOG at different levels had no significant effect on moisture and ash contents of carcass meat comparing to the control. The values of moisture and ash ranged from 60.00 to 62.10% and 4.05 to 4.20% for moisture and ash contents,

Table 4: Effect of Effect of adding different levels of LOG on chemical composition of carcass meat of experimental groups

	Experimental groups				
Item	G_1 Basal ration	G_2 10 ml LOG / kg feed	G_3 20 ml LOG / kg feed	G_4 30 ml LOG / kg feed	SEM
Moisture (%)	61.80	60.00	62.10	61.40	0.45
Chemical analysis (%) on DM basis					
Crude protein (CP)	92.05[b]	92.55[a]	92.69[a]	92.84[a]	0.10
Ether extract (EE)	3.75[a]	3.30[b]	3.21[b]	3.11[b]	0.09
Ash	4.20	4.15	4.10	4.05	0.03

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

SEM: standard error of the mean.

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

respectively among the four experimental groups. On the other hand, it significantly (P<0.05) increased their values of crude protein, meanwhile, it significantly (P<0.05) decreased their content of fat in carcass meat.

The data concerning of meat quality near from the results that found by Onibi *et al.* [56] who evaluated, the moisture and lipid contents of meat from the broiler chickens that fed diets contained (0; 500 or 5, 000 mg/kg diet. They noted that, moisture contents of the meat were not significantly (P>0.05) affected by garlic supplementation, muscle type and interaction of the two factors. Also, the value of the moisture contents of the meat did not follow any trend in relation to treatment groups or muscle type. Meanwhile, Gardzielewska *et al.* [74] showed that broiler chickens feed supplementation with garlic (*A. sativum*) was no significant (P>0.05) effect on dry matter content of the muscles. Onibi *et al.* [56] reported that, the moisture contents of the meat was (73.13-76.64 % of fresh meat). Meanwhile, Gardzielewska *et al.* [74] recorded that the moisture contents of the meat from broiler chickens on plant supplemented feed (74.70-75.0.3 % of fresh meat). Onibi *et al.* [56] observed that the lipid contents of the meat was significantly (P<0.01) affected by the muscle type but not by garlic supplementation or the interaction of garlic supplementation and by 43% compared with the control group. Also, the thigh muscle had the highest lipid content (82.94 g/kg), followed by drumstick muscle (66.90 g/kg) and the lowest was recorded for breast muscle (49.08 g/kg). Also, Ikeme [75] and Onibi [76] noticed that, fat deposition has been reported to be higher in thigh than drumstick and lowest for breast muscle.

Amino Acids Profile of Carcass Meat: Data of Table (5) cleared that except for the isoleucine the others essential amino acid profile was affected by inclusion LOG in broiler chicks rations. Meanwhile, except for the aspartic the other non-essential amino acid profile was affected by inclusion LOG in broiler chicks rations. The best values of essential amino acid includes (histidine, leucine and lysine) was recorded by broiler chicks fed 10 ml LOG/ kg feed (G_2) followed by (arginine, methionine and phenylalanine) that recorded by broiler chicks fed 30 ml LOG/ kg feed (G_4). Chemical composition of chicken meat is important and usually determines the possibilities for storage or further processing. Regarding the influence of synbiotic on the amino acids composition of broiler muscles, the available information is scarce and thus the discussion of this topic is somewhat difficult. Salah *et al.* [77] noted that the essential amino acids (EAA) contents of the breast and thigh muscles, the levels of leucine and methionine were significantly higher in the group fed diet supplemented with the synbiotic compared with the control group. Among the nonessential amino acids (NEAA) contents of breast and thigh muscles, the concentration of histidine was significantly greater in the symbiotic supplemented group in comparison with the control group. However, no significant variation was noted across the different experimental groups regarding the contents of serine, glutamine, arginine and aspartic acid in broiler muscles. However, the effects of probiotic supplements on the amino acids contents of broiler muscles have been mentioned in some trials [78, 79]. Mostly, the dietary synbiotic supplements increased the contents of some essential (leucine, isoleucine, lysine, methionine and histidine) and nonessential (arginine and tyrosine) amino acids in breast and thigh muscles. This may attributed to the improvement of protein solubility and emulsifying capacity of sarcoplasmic protein of breast muscles in broiler chickens as noted by Kim *et al.* [80]. Others reported that probiotics based supplements improved the protein contents and the respective contents of essential amino acids [81]. Mahmood *et al.* [82] noted that the dietary supplementation with probiotics improved the protein contents of broiler muscles. Additionally, Abdulwahab and Horniakova [78] reported that dietary supplementation with *Lactobacillus* spp. improved the

Table 5: Effect of Effect of adding level of LOG on amino acids profile of carcass meat of experimental groups

	Experimental groups				
Amino acids profile	G_1 Basal ration	$G_2$10 ml LOG / kg feed	$G_3$20 ml LOG / kg feed	$G_4$30 ml LOG / kg feed	SEM
1-Essential amino acids					
Arginine	4.48^{b}	4.35^{c}	4.30^{c}	4.63^{a}	0.042
Histidine	3.41^{a}	3.43^{a}	3.33^{ab}	3.21^{b}	0.033
Isoleucine	315	3.23	3.21	3.22	0.022
Leucine	4.65^{b}	4.86^{a}	4.77^{ab}	4.73^{ab}	0.034
Lysine	5.16^{c}	5.45^{ca}	5.31^{b}	5.26^{b}	0.034
Methionine	2.17^{b}	2.10^{c}	2.10^{c}	2.32^{a}	0.028
Phenylalanine	2.77^{b}	2.81^{ab}	2.76^{b}	2.92^{a}	0.026
Theronine	2.94^{bc}	2.86^{c}	3.10^{ab}	3.22^{a}	0.048
Valine	2.86^{b}	3.02^{a}	2.95^{a}	2.84^{b}	0.025
2-Non essential amino acids					
Aspartic	5.80	6.10	6.14	6.04	0.064
Serine	2.25^{c}	2.42^{bc}	2.63^{ab}	2.67^{a}	0.058
Cystine	1.76^{a}	1.87^{a}	1.31^{c}	1.56^{b}	0.067
Glutamic	5.96^{ab}	6.09^{a}	5.97^{ab}	5.87^{b}	0.033
Glycine	2.63^{a}	2.51^{b}	2.53^{ab}	2.44^{a}	0.025
Alanine	3.32^{ab}	3.38^{a}	3.32^{ab}	3.28^{b}	0.016
Tyrosine	2.22^{c}	2.33^{bc}	2.42^{ab}	2.48^{a}	0.033
Proline	2.62^{a}	2.36^{b}	2.40^{b}	2.55^{a}	0.035

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

SEM: standard error of the mean.

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

contents of essential (isoleucine, threonine, methionine and histidine) and nonessential (glycine and tyrosine) amino acids of breast and thigh muscles in Ross broilers. In a trial carried out by Podolian [79] showed that the addition of probiotic mixture to the broiler diets could improve the contents of lysine, arginine and methionine in the breast muscles. However, Mehdipour *et al.* [83] recorded no positive changes in the physicochemical characteristics of thigh muscles when quails were fed on a diet supplemented with synbiotic. Moreover, others recorded that the supplementation of probiotics reduced the levels of isoleucine and leucine in pectoral and thigh muscles of broilers [79]. These conflicted outcomes may be attributed to a clear variation in the breed, duration of dietary supplementation, dose and other managerial aspects.

Fatty acids profile of carcass meat: Data presented in Table (6) showed that incorporation LOG at different levels (0, 10, 20 and 30 ml of LOG/ kg feed had no significant effect (P>0.05) on all fatty acids profile of carcass meat determined. Dietary treatments in significantly decreased the values of saturated fatty acids (SFA). The corresponding values of SFA of carcass meat were 39.98, 33.30, 37.14 and 36.91 for G_1, G_2, G_3 and G_4, respectively. Meanwhile mono unsaturated fatty acids (MUFA); Poly unsaturated fatty acids (PUFA) and total unsaturated fatty acids (TUFA) of carcass meat were in significantly (P>0.05) increased with incorporation LOG in broiler rations. The best value of TUFS (66.70%) was recorded by G_2 that received 10 ml LOG/kg feed, followed by (63.09%) for G_4 that received 30 ml LOG/kg feed. Meanwhile, the value of TUFS of carcass meat for broiler chickens that received 20 ml LOG/kg feed (G_3) was (62.86%). Lipids and their derivative fatty acids are present in muscles as structural components of muscle membranes, as storage droplets of triacylglycerol between muscle fibers and as adipose tissue. The form and nature of these fatty acids decide color stability, drip loss and the development of oxidative rancidity, which ultimately decide the sensory and nutritional quality of meat products [84-87]. The lipid oxidation rate is directly proportional to the un-saturation of fatty acids, which ultimately decides the color and oxidative stability of meat products [88, 89]. On the other hand, Mariutti *et al.* [90] studied the effect of the addition of sage and garlic on lipid and cholesterol oxidation in chicken meat, in the presence of salt as prooxidant. They noted that the content of unsaturated fatty acids did not change in the presence of sage; on the contrary, with garlic, the contents of these fatty acids were decreased after cooking and storage. The hexanal and pentanal contents were lower in chicken patties containing sage and they were higher in patties with garlic.

Table 6: Effect of Effect of adding different levels of LOG on fatty acids profile of carcass meat of experimental groups

Fatty acids profile		Experimental groups			
		G_1 Basal ration	$G_2$10 ml LOG / kg feed	$G_3$20 ml LOG / kg feed	$G_4$30 ml LOG / kg feed
C14: 0	Myristic acid	0.66	0.46	0.62	0.56
C14: 1 ω 5	5- tetradecenoic acid (Phtosteric)	0.90	0.35	0.32	0.20
C15: 0	Pentadecanoic acid	0.00	0.00	1.66	1.41
C16: 0	Palmitic acid	29.67	25.5	25.09	25.29
C16: 1 ω 9	Palmitoleic acid	4.40	0.50	0.19	0.53
C16: 1 ω 7		0.00	5.50	4.14	4.44
C17: 0		0.00	0.00	0.24	0.00
C18: 0	Stearic acid	9.65	7.34	9.53	9.42
C18: 1 ω 9	Oleic acid	39.99	42.71	37.44	42.46
C18: 1 ω 7	Vaccinic acid	2.00	2.04	1.76	2.01
C18: 1 ω 5	Octadecosaenoic acid	0.90	0.30	0.47	0.29
C18: 2 ω 5		0.00	1.00	1.13	0.29
C18: 2 ω 6	Linoleic acid	8.71	11.34	8.60	6.32
C18: 2 ω 4		0.00	0.00	0.19	0.98
C18: 3	Gama linolenic acid	0.00	0.31	0.00	0.00
C18: 4 ω 3	Alpha 9, 11, 13, 15–octadecatetraenoic acid	0.00	0.00	0.31	0.00
C20: 0	Arachidic acid	0.00	0.00	0.00	0.23
C20: 1 ω 11	Eicosaenoic acid	0.00	0.00	0.31	0.46
C20: 1 ω 9	Gadoleic acid	0.00	0.47	0.68	0.68
C20: 1 ω 7	Cis–9–eicosaenoic acid	0.76	0.34	1.02	1.03
C20: 1 ω 5	Ecosaenoic acid	1.35	0.68	0.82	0.94
C20: 4 ω 3	Arachidonic acid	0.66	0.43	1.75	1.92
C20: 5 ω 3	EPA	0.35	0.41	0.61	0.24
C22: 1 ω 11	Cis–11–decosenoic (Cetoleic)	0.00	0.00	0.87	0.00
C22: 1 ω 9	Erucic acid	0.00	0.00	0.89	0.00
C22: 1 ω 7		0.00	0.00	0.15	0.00
Non identified		0.00	0.32	1.21	0.30
Saturated fatty acids (SFA)		39.98	33.30	37.14	36.91
Mono unsaturated fatty acids (MUFA)		50.30	52.89	49.06	53.04
Poly unsaturated fatty acids (PUFA)		9.72	13.81	13.80	10.05
ω 3		1.01	0.84	2.67	2.16
ω 4		0.00	0.00	0.19	0.98
ω 5		3.15	2.33	2.74	1.72
ω 6		8.71	11.65	8.60	6.32
ω 7		2.76	7.88	7.07	7.48
ω 9		44.39	43.68	39.2	43.67
ω 11		0.00	0.00	1.18	0.46
Others		0.00	0.32	1.21	0.30
Total Unsaturated fatty acids (TUFA)		60.02	66.70	62.86	63.09

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

Blood Parameters: Biochemical and hematological blood parameters: Data of Table (7) mentioned that inclusion LOG in broiler chicks in significantly decreased the determined values of total protein content. Meanwhile, albumin and globulins contents were in significantly increased. Creatinine values were decreased with increasing the level adding of LOG. Uric acid values was not affected by incorporation LOG in broilers chickens rations (values ranged from 2.29 to 2.75 among the four groups). Total cholesterol, triglycerides, GOT and GOT values were decreased with increasing the level supplementation of LOG. On the other hand, adding LOG at different levels had no significant effect on all evaluated hematological blood parameters that {includes hemoglobin concentration, erythrocyte counts (RBC's), leukocyte number (WBC's) and deferential leucocyte count that includes (heterocytes count, lymphocytes, eosinophils, monocytes and basophils}.

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

Table 7: Effect of adding different levels of LOG on some biochemical and hematological blood parameters of the experimental groups

Item	Experimental groups				SEM
	G_1 Basal ration	$G_2$10 ml LOG / kg feed	$G_3$20 ml LOG / kg feed	$G_4$30 ml LOG / kg feed	
1-Biochemical blood parameters					
Total proteins (g/dl)	3.15	3.42	3.35	3.30	0.082
Albumin (g/dl)	1.30	1.43	1.40	1.35	0.036
Globulins (g/dl)	1.85	1.99	1.95	1.95	0.057
Albumin/ globulins ratio	0.70	0.72	0.72	0.69	0.019
Creatinine (mg/dl)	0.23^{a}	0.20^{ab}	0.19^{ab}	0.18^{b}	0.008
Uric acid (mg/dl)	2.29	2.47	2.69	2.75	0.17
Total cholesterol (mg/dl)	120.6	118.5	116.0	114.0	3.13
Triglycerides (mg/dl)	56.63^{a}	54.48^{a}	48.60^{ab}	42.80^{b}	3.47
GOT (U/l)	87.25^{a}	85.75^{a}	80.50^{ab}	71.50^{b}	3.36
GPT (U/l)	20.25	19.50	18.75	17.00	0.92
2-Hematological blood parameters					
Hemoglobin concentration (Hb), (g/dl)	11.95	10.25	11.70	10.25	0.35
Erythrocyte counts (RBC's), ($x10^6/mm^3$)	4.09	3.72	3.86	3.87	0.08
Leukocyte number (WBC's), (x 10^3/ mm^3)	31.23	27.21	29.00	29.28	0.89
Deferential leucocyte count					
Heterocytes count, (x 10^3/ mm^3)	15.50	18.50	19.50	20.25	0.95
Lymphocytes, ($x10^3$/ mm^3)	79.00	76.75	74.75	74.00	1.06
Eosinophils, ($x10^3$/ mm^3)	2.25	1.75	2.75	1.75	0.22
Monocytes, ($x10^3$/ mm^3)	3.00	3.00	2.75	3.50	0.34
Basophils, ($x10^3$/ mm^3)	0.25	0.00	0.25	0.50	0.15

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

SEM: standard error of the mean. GOT: Glutamic oxaloacetic transaminase.

GPT: Glutamic pyruvic transaminase. RBC's: Red blood cell count.

WBC's: White blood cell count.

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

and antiperoxide activity [68]. These compounds are related to a decrease in blood lipid, liver protein and glucose [91, 92]. Goodarzi *et al*. [26] reported that the use of onion bulbs in the broiler diet can lower triglycerides and total cholesterol in blood serum. Allicin and its derivative compounds are the primary active substances responsible for the hypolipidemic and hypocholesterolemic effects of onion and garlic [68, 93]. These compounds possibly impact hypercholesterolemia by inhibiting hepatic cholesterol biosynthesis, enhancing cholesterol turnover to bile acids [94] or inhibiting cholesterol absorption from the intestinal lumen [95]. In addition to, An *et al*. [5] fed White mini broilers diets contained 0, 0.3 or 0.5% of onion extract, they noted that there were no significant differences in the activities of GOT and GPT in serum among groups. Meanwhile, El-Demerdash *et al*. [96] found that inclusion onion juice increased levels of both GOT and GPT could restore to normal levels in alloxan diabetic rats. They also, showed that onion juice exerted antioxidant effects and alleviated the tissue damage caused by alloxan-induced diabetes. In a previous study, noticed that significant positive effect on antioxidant activity was shown with onion extract [97]. The measurement of serum GOT and GPT activities indicative of tissue damage in bird is also a valuable tool to determine a safe inclusion level for non-conventional feedstuff and new additives [98]. On the other hand, An *et al*. [5] 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. Also, they found 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. It is well known that dietary onion effectively lowers serum cholesterol levels in experimental rats as well as in humans. The decreased levels of blood cholesterol have been reported in rats fed normal diets with onion [99] and high sucrose diet with onion essential oil [100]. Hypocholesterolemic effects with dietary onion may be attributed to decreased cholesterol secretion from liver, or to increased uptake of high density lipoprotein (HDL) uptake into liver An *et al*. [5]. In addition to, Goodarzi *et al.* [26] noticed that broilers fed diet contained 3% onion had a significant higher HDL and lower blood triacylglycerol levels than those of control. Also, Sklan *et al*. [101] found no significant difference in

hepatic cholesterol in chicks fed diet with onion. Moreover, Aditya *et al*. [102] reported that when broiler chicks fed diets contained 5, 7.5 or 10 g onion extract (OE)/ kg feed was not affected on total cholesterol, high-density lipoprotein- cholesterol (HDL-cholesterol), triglyceride and glucose. Also, in a study carried out by Goodarzi *et al*. [26] noted that broiler diet did not exert any change on serum total cholesterol. However, other studies reported a significant decreasing in serum total cholesterol by dietary quercetin [103] and onion extract [5].

CONCLUSION

Under conditions similar to those obtained in the present study, it can be mentioned that, natural bioactive mixture composed of lemon, onion and garlic juice (LOG) can be used safely in broilers chickens rations up to 30 ml LOG/ kg feed to improve their growth performance, with out causing any adverse effect on their carcass characteristics, meat quality includes (amino acids and fatty acids profiles) and decreased values of total cholesterol, triglycerides, GOT and GOT.

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