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IMPACT OF DIETARY SUPPLEMENTATION WITH DIFFERENT HERB SPECIES ON BODY PERFORMANCE MEAT QUALITY AND HEALTH STATUS IN BROILER CHICKENS

ABSTRACT OF A DISSERTATION

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The dissertation contains 165 pages of text, 10 tables and 30 figures. The sources used include 408 titles.

The dissertation is divided into 5 sections:

I. Introduction
II. Purposes and tasks
III. Materials and methods
IV. Results and discussion
V. Work Contributions

The defense of the dissertation will be held at 1.30 p.m. on June 14, 2024, in the „Acad. Mako Dakov" hall of the University of Forestry - Sofia, 10 Kliment Ohridski Blvd at an open meeting of the Scientific Jury composed of:

Chairman: Assoc.Prof. Metodi Hristov Petrichev, DVM, PhD

Members: Prof. Hristo Yordanov Daskalov, DVM, PhD
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The materials on the defense (dissertation, abstract, reviews and opinions) are available to those interested on the website of the University of Forestry (www.ltu.bg) and in the dean's office of FELA - Sofia, Blvd. Kliment Ohridski "№10.

I. INTRODUCTION

Since the 1960s, global production of poultry meat has been growing faster than any other meats. The growth is particularly strong in broiler production in the developing countries in recent years (Taha, 2003). The significant growth in poultry (especially broiler chicken) production and consumption in the developing countries has important implications for the global trade for all meat products, as well as feeds and other related inputs (Landes *et al.*, 2004; Taha, 2003). Despite its many advantages and the positive market outlook, the world broiler sector faces increasing challenges (Shane, 2003). One of these is the increasing consumer concerns over food safety, animal welfare, product quality, and environmental issues associated with industrialized poultry production systems. Poultry production in Lebanon was one of the major components of the Lebanese agricultural sector. During the late 60's and the 70's, the poultry industry was one of the largest and most advanced in the Middle East. Currently, about 450,000 breeding hens produce about 2,500,000 layers and about 65,000,000 broilers per year. There are also small ventures in quail, duck, and turkey production. Exports are limited as most of the Arab states produce the major portion of their needs while imports are mainly from countries like Brazil, Thailand, and China where production costs are much lower than Lebanon. The poultry industry depends on imported feed except for the locally produced minor elements: Sodium, Calcium, and Chlorine. These do not represent more than 8% of the feed. All other ingredients such as Soybean, Maize, Barley, sunflower meal, peanut meal, animal proteins, vitamins, mineral salts, and other feed additives such as growth promoters and enzymes are imported. With the absence of control mechanisms and facilities, feed quality seriously affects quality and quantity of production (Darwish, 2003). Feed additives have a great influence on the performance of broiler chicken and feed efficiency. Approximately 80% of domestic animals have been fed synthetic compounds for the purpose of medication or growth promotion. After the risks and concerns from the use of antibiotics in animal feeding, and the banning of the use of those chemicals as growth promoters, investigation was led to find a new natural alternative that can be used as feed additives. After the low levels of antibiotics used in animal feed as growth promoters has generated an antibiotic-resistant population of bacteria, the use of the antibiotics in broiler production has been limited, and the use of plant origin substitutes to the chemical products is getting more and more attention. Fungal contaminants producing mycotoxins, or merely affecting the nutrient content of feedstuffs whether protein, amino acids, fiber, or energy could cause severe production losses. Most producers rely on theoretical information regarding the composition of feedstuffs, especially with amino acids and energy content (FAO, 2000). High poultry feed costs as well as high mortality rates in the poultry sector remain a major constraint to expanding broiler production in the Middle East. Endemic poultry diseases have increased chicken mortality rates in many poultry farms in recent years, which have been estimated at 25 %, on average, and reached 50 % in some individual farms (FAO, 2010). Iji *et al.* (2001) and Issa and

Abo Omar (2012) noted that the use of antibiotics as growth promoters is facing serious criticism. There are some important reasons that restrict the use of antibiotics such as the drug resistance in bacteria and the drug residues in meat (CAFA, 1997). To overcome the poor performance and the increase susceptibility to diseases resulted from removal of antibiotics from birds' diets, attempts were made to find other alternatives. The utilization of growth promoters of natural origin become of an interest in recent years Iji *et al.* (2001). Alternatives to antibiotic growth promoters used in poultry production includes herbs and many other plant sources and extracts (Ozogul et al., 2015; Gracia et al., 2016; Yang et al., 2018). Aside from antibiotic activities, plant extracts do stimulate the secretion of digestive enzymes thereby improving digestion and making nutrients in feeds readily available for absorption (Chao et al., 2000). These synergic activities by plants' active substances, may improve health and performance of livestock (Manzanilla et al., 2001). Odoemelam *et al.* (2013) observed that there are strong indications that herbs, spices, and their products exert antioxidative, antimicrobial and growth promoting effects in livestock. The antioxidative efficacy of some of the herbs and spices in protecting the quality of feed as well as that of food derived from animals fed these substances cannot be ruled out. For antimicrobial actions, observations in vivo support the assumption that they possess the potential to contribute to the final reduction of intestinal pathogen pressure. This action compares with the role played by some other antimicrobial feed additives and organic acids. Furthermore, studies reveal that they contribute to an enhanced digestive enzymatic activity and absorption capacity. Soccol *et al.* (2010) added that they also stimulate intestinal mucus production, which contributes to relieve pathogen pressure through inhibition of adherence to the mucosa. However, products containing blends or mixtures of phytogenic compounds appear to produce these results. Iji *et al.*, (2001) stated that in pursuit of improved chicken healthiness and to fulfill consumer expectations in relation to food quality, poultry producers more and more commonly apply natural feeding supplements, mainly herbs. Their antibiotic potential effects, growth promoting, and availability are the most beneficial parts of herbs, which have drawn the attention themselves. Consequently, there is a need for a systematic approach to explain the efficacy and mode of action of herbs and spices. There should also be studies to show the possible interaction of these plant materials with other feed ingredients and to show their potential in substituting conventional antibiotics used in the poultry industry to find sustainable and efficient solutions to the farmers.

II. PURPOSES AND TASKS

Main goal: the main goal of this study is to investigate the potential of local natural herbs (Thyme, Peppermint, Rosemary, Chamomile, Garlic powder, and Onion powder) as growth promoters and immunity boosters of Ross broiler chicken targeting the substitution of antibiotics conventionally used in the Lebanese broiler industry. Therefore, to evaluate the impacts of tested natural herbs on broiler physiology and growth, of tested natural herbs on broiler meat production and quality and of tested natural herbs on broiler serum biochemistry profile, hematological indices, and immunology

The tasks consisted in conducting a first trial where 8 groups of broiler chicken are fed by rosemary, chamomile, peppermint, and thyme as supplements to the basal diets to evaluate their effects on health status, body weight, feeding and feed conversion ratio, carcass yield, weight of internal organs, meat quality indicators, conducting a second trial where 5 groups of broiler chicken are fed by garlic and onion powders as supplements to the basal diets to evaluate their effects on health status, body weight, feeding and feed conversion ratio, carcass yield, weight of internal organs, meat quality indicators, assessing the blood parameters, serum biochemistry profile and immunology, evaluating and comparing obtained results of both trials and drawing conclusions on potential alternatives for conventional antibiotics based chicken physiology, immunity and meat quality.

III. MATERIALS AND METHODS

1. Experimental Setup

1.1. Experimental Site and Distribution

TRIAL I (Peppermint-Thyme-Chamomile-Rosemary)

An experiment was conducted during for 31 days (starting-growing period) in an opened poultry house with windows on both facing walls at Bekaa valley at Al Hassan poultry farm to study the impact of feeding broiler chicks' basal rations free of antibiotics and antioxidants mixed with peppermint, thyme, chamomile and rosemary and their combination on body performance during the growth period. Feeding of natural herbs started after 20 days from the initiation of the experiment. A small broiler flock of 432-one-day old, unsexed Ross chicks was obtained from a commercial hatchery and reared under one roof in the same environmental and productive conditions in floor pens, with wood shavings (7-10 cm thickness), with natural ventilation through windows and roof. They were divided and randomly distributed among eight separated groups and fed starter basal diets based on yellow corn-soybean meal mixture mash. All chicks received the same starter basal ration for 19 days (starting period) with neither addition of antibiotics and antioxidants nor herbs and spices. From day one to ten of age, chicks were placed under artificial gas brooder located at a height not exceeding 1 m above floor level. Circular cardboard guard of 40 cm height was used to keep the birds inside. Each group was provided approximately with 5-m2 floor space. All groups were receiving continuous light of 24 hours. The house was supplied with 75- Watt Tungsten pulp lamps and adjusted to achieve light intensity between 5-10 lux at floor level. At age of 20 days, each group (CGIaNegative, CGIIaPositive, EGIII, EGIV, EGV, EGVI, EGVII and EGVIII) was subdivided into three replicates (R1, R2 and R3) where each group/replicate consisted of 54 chicks and fed for 12 days (Growth period).

Note that the average values of the three replicates of each group were statistically calculated and considered as a result for each group.

- Control group Ia (CG Ia Negative control of 1st trial) was fed basal diet without antibiotics and antioxidants
- Group IIa (CG IIa Positive control of 1st trial) was fed basal diet with antibiotics (15 mg of virginiamycin/kg as proposed by Goodarzi et. al. (2013) and antioxidant (125 mg/kg, ethoxyquin).
- Group III (EG III Peppermint) was fed the same basal diet as for negative control group CG Ia supplied with 10g/kg peppermint meal.
- Group IV (EG IV Thyme) was fed the same basal diet as for negative control group CG Ia supplied with 10g/kg thyme meal.
- Group V (EG V Peppermint + Thyme) was fed the same basal diet as for negative control group CD Ia supplied with a mixture of 5g/kg peppermint and 5g/kg thyme meals.
- Group VI (EG VI Rosemary) was fed the same basal diet as for negative control group CG Ia supplied with 10g/kg rosemary dry meal.
- Group VII (EG VII Chamomile) was fed the same basal diet as for negative control group CG Ia supplied with 10g/kg chamomile flowers dry meal.
- Group VIII (EG VIII Rosemary + Chamomile) was fed the same basal diet as for negative control group CG Ia supplied with a mixture of 5g/kg rosemary dry meal and 5g/kg chamomile flowers dry meals.

TRIAL II (Garlic Powder-Onion powder)

At Al-Labweh village, a total of 270 Ross line one-day-old broiler chickens were used in the feeding trial that lasted until the birds reached 31 days of age - Slaughter age. In total 4 floor pens each of 4 m2 surface area covered with wood shaving (5- 7 cm thickness) were used, each containing 54 chicks. At age of 20 days, each group (CG Ib Negative, CG IIb Positive, EG IX, EG X, and EG XI) was subdivided into three replicates (R1, R2 and R3) where each group/replicate consisted of 55 chicks and fed for 12 days (Growth period). Note that the average values of the three replicates of each group was statistically calculated and considered as a result for each group.

- Control group I (CG Ib Negative control of 2nd trial) was fed basal diet without antibiotics and antioxidants
- Group II (CG IIb Positive control of 2nd trial) was fed basal diet with antibiotics (15 mg of virginiamycin/kg as proposed by Goodarzi et. al. (2013) and antioxidant (125 mg/kg, ethoxyquin).
- Group IX (EG IX Onion) was fed the same basal diet as for negative control group CG Ib supplied with 10g/kg onion powder.

• Group X (EG X Garlic) was fed the same basal diet as for negative control group CG Ib supplied with 10g/kg garlic powder.
• Group XI (EG XI Onion + Garlic) was fed the same basal diet as for negative control group CG Ib supplied with a mixture of 5g/kg onion powder and 5g/kg garlic powder meals.

Note that negative control (CG Ia; CG Ib) and positive control (CG IIa; CG IIb) diets, are mixtures with the same ingredients and nutritive value fed to broilers in both trials whereas, IIa and IIb were supplied with antibiotics and antioxidants.

1.2. Feeding.

During both trials, the birds were given ad libitum access to feed and water.All diets were formulated to meet minimum nutrient requirements established by the NRC (1994). The chicks were fed purchased commercial broiler starter diet (230 g CP/kg and 13.1 MJ ME/kg) from day 1 to 19, a commercial broiler grower diet (210 g CP/kg and 13.4 MJ ME/kg) from day 20 to 31 (Labeled and guaranteed by the manufacturer). Vitamin Premix was provided to basic diet (per kg of diet): vitamin A, 5,500 IU; vitamin D3, 1,100 IU; vitamin E, 11 IU; vitamin B12, 0.0066 mg; riboflavin, 4.4 mg; niacin, 44 mg; pantothenic acid (calcium pantothenate), 11 mg; choline (choline chloride), 190.96 mg; menadione, 1.1 mg; folic acid, 0.55 mg; pyridoxine (pyridoxine hydrochloride), 2.2 mg; biotin, 0.11 mg; thiamine (thiamine mononitrate), 2.2 mg; ethoxyquin, 125 mg; 15 mg of virginiamycin (antibiotic). Mineral Premix was provided to basic diet (in mg/kg of diet): Mn, 120; Zn, 100; Fe, 60; Cu, 10; I, 0.46; Ca, minimum: 150, maximum: 180. Clean drinking water and the experimental rations were available ad libitum in troughs during the entire experiment (Fig.1). The chicks were fed starter diet from one to 19 days and grower from 20 days until slaughter (31 days). All diets fed were in the form of mash, formulated and prepared commercially to be isocaloric and isoproteinic, purchased at the beginning of the experiment.

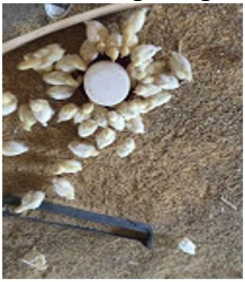

Figure 1. Water and feeding troughs

2. Vaccination program.

All birds were vaccinated against Gumboro (at 10 and 20 days of age) Infectious bronchitis (at 15 days of age) and Newcastle diseases (at 20 days of age).

3. **Health and mortality.**

General health status of the birds was checked daily, and number of dead chicks was recorded in case of mortality.

4. **Body weight measurements.**

Measurement of one-day old chick was recorded using electronic balances (0.00g). At 19 days of age average live body weight was calculated by weighing from each group 3 batches by 3 birds each, where the average from each batch was recorded and then the total average of the 3 batches was registered. At 31 days of age all birds were left to fast for 24 hours and then from every replicate 3 batches by 3 birds were weighed before and after slaughter where they were left to completely bleed after which the feather was manually plucked, and the troops eviscerated to get net weight. Breasts (left and right) were cut and then heart and liver removed and weighed alone.

5. **Feed intake (FI), live body weight gain (LBWG) and feed conversion ratio (FCR)**

Live body weight (LBW). Live body weight (LBW) and feed intake (FI) were recorded every time feeding phase was changed using a digital balance (± 0.01g) where FCR was calculated.

FCR= FI/ LBW

Live body weight gain (LBWG). Butcher and Nilipour (2004) stated that the average gram daily weight gains play an important role in the optimum growth of the birds. Under normal practical conditions, a broiler must gain an average of 65 grams or more per day. The average daily weight gain is not uniform for each week and varies considerably depending on age and sex.

Feed intake (FI). Feed intake and rate of body weight gain were determined at the end of each feeding period (19 and 31 days of age) where FCR was calculated. With feed accounting for up to 70% of total production cost, efficiency of use is an important factor in broilers performance. To determine optimum nutrient concentrations in broiler diets, feed intake of broilers varying in body weight should be properly predicted. There are many factors which affect feed intake of poultry, these include: breed or strain, age, nutrient balance of the diet, ambient temperature, health and welfare status of the birds, accessibility of the feed, flock density. In addition, certain feed ingredients, poor feed quality or feed contamination can have adverse effects on feed intake due to poor palatability or the presence of toxic factors. A broiler chicken will eat about 1 kg of starter, 1.5 kg of grower and 1.5 kg of finisher to reach market weight.

Feed conversion ratio (FCR). Feed conversion ratio is a measure of how well a broiler flock converts feed intake into live weight and provides an indicator of management performance, and profit at any given feed cost. As feed costs represent 60-70% of the total cost of broiler production, the efficient conversion of feed into live weight is essential for profitability, and small changes in FCR at any given feed price can have a substantial impact on financial margins. The FCR of broiler chicken has been improved from 3.5 in 1953 to about 1.7 or less in 2008 (Ghosh, 2015). With the recent rise in feed costs internationally and forecasts, that cost will remain high; the bird's ability to convert nutrients is a particularly important aspect of overall performance efficiency.

6. Carcass characteristics and meat quality

6.1. Carcass characteristics

Ten birds having body weight near the corresponding group average weight were slaughtered at 33 days of age for carcass and meat quality measurements. Following plucking and evisceration, carcasses and visceral organs (liver, heart, gizzard and proventriculus) were weighed using a digital balance (±0.01g). Each carcass was identified, suspended in the air for 20 minutes at ambient temperature (+24◦C) and conserved at 4◦C for carcass and meat quality measurements. Twenty-four hours *post mortem*, carcasses were dried with filter paper and reweighed. Breast and thigh muscles were removed from carcasses and weighed (±0.01g).

Carcass, breast, and thigh muscles yield (%) were calculated.

Carcass yield (%) = (Carcass weight/ live body weight) *100

Muscle yield (%) = (Muscle weight/carcass weight) * 100

6.2. Meat quality measurements

Postmortem muscle pH, color, and drip loss (%) were performed on the left scallops, whereas right scallops were frozen to measure pH, color and drip loss, thawing and cooking losses (%) and to estimate the tenderness of meat.

6.2.1. Postmortem breast muscle pH

Breast pH was measured at two-time interval, 24 and 48-hour postmortem, using a portable pH meter (Adwa AD 131 Ph/mV Meter), equipped with a Piercing Tip Micro Probe. The pH meter was calibrated at pH 4.0 and pH 7.0 prior to use. Each sample was measured in triplicate by a direct insertion of the probe at 1-inch-deep in the muscle and the average pH value calculated for each treatment. The electrode was washed with distilled water after each use (Fig. 2).

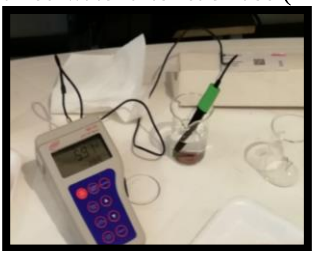

Figure 2. pH measurements

6.2.2. Meat color

A whole piece of breast meat was evaluated for color in 3 replicates at 24 and 48 hours using the Hunter Lab colorimeter (ADCI-60-C instrument). The color of the breast meat was evaluated using the CIE color system (Fig. 3), including L* (lightness), a* (greenness and redness), and b* (blueness and yellowness). All measurements were carried out on the surface of the scallop, in an area free of color defects (bruises, blood spots and hemorrhages).

Figure 3. Color measurements

6.2.3. Water holding capacity (WHC)

6.2.3.1. Drip loss

Left raw breast muscles were individually weighed at 24 hours postmortem, then they were reweighed after blotting dry by paper towel. The samples were then placed in sealed polyethylene plastic bags, vacuum-packaged and stored in a chiller at 4°C. The same procedure was repeated at 48 hours *postmortem* and the drip loss was calculated as a percentage relative to the initial muscle weight. Drip loss (%) = [(Weight before treatment - weight after treatment)/ weight before treatment] * 100.

6.2.3.2. Thawing and cooking losses

Left scallops were packed in polyethylene bags and put in a refrigerator + 4 °C for 24 hours while the right scallops were frozen for 1 month to determine thawing and cooking losses. After 24 hours the left scallops were taken from bags, dried with filter paper and reweighted. The difference in the initial and final weights was calculated for the percentage of thawing loss.

Thawing loss (%) = [(weight before freezing – weight after thawing)/ weight before freezing] *100.

After thawing loss, meat was used for cooking. Aaslyng *et al.* (2003) complained that cooking loss is a combination of liquid and soluble matters lost from meat during cooking. Cooking loss is an important factor in meat industry as it determines the technological yield of the cooking process (Kondjoyan *et al.*, 2013). Nutritionally, cooking loss brought about loss of soluble proteins, vitamins and different supplements (Yarmand *et al.*, 2013). Samples were packed in polyethylene bags, cooked in a pre-heated water bath at 80◦C for 15 minutes, cooled, dried with filter paper, and weighed. Cooking loss was calculated as the difference in weight between fresh and cooked samples with respect to fresh weight.

Cooking loss (%) = [(Weight before cooking - Weight after cooking)/Weight before cooking] * 100.

6.2.4. Estimation of meat tenderness

Meat tenderness was estimated using a penetrometer (interface RS232C) with a needle of 2.5 g based on a weight of 47.5 g, thus attaining a total weight of 50 g. cooked samples were cooled to room temperature and one strip (3 cm x 1 cm x 0.5 cm) was cut from the center of each fillet. The strip was placed on a horizontal support and the force of the needle was applied perpendicular to the longitudinal direction of the fibers (Sazili *et al.*, 2005). The penetrometer needle depth (PND; in mm) was recorded and an average of 3 replications by sample was calculated (Fig. 4).

Figure 4*:* Penetrometer

7. Blood analysis

7.1. Blood Collection

Blood was collected at the end of each trial (at slaughter). Ten mls of blood was collected from the neck vein of ten birds per broiler group. Two mls of the blood was released into tubes containing anti-coagulant Ethylene diamine tetra-acetate (EDTA) for hematological parameters, five mls of blood samples meant for serum analysis were released into plain bottles and three mls collected in the heparinized tubes for plasma.

7.2. Haematological indices (miss some indices like RBC and others)

Haematological indices which include Haemoglobin concentration (Hb), Red Blood Cells (RBC) were determined using van slyke apparatus, Pack Cell Volume (PCV) – hacksley haematocrit centrifuge (UK), White Blood Cell (WBC), WBC differentials (neutrophils, lymphocytes, monocytes, eosinophils and basophils) as determined using the Neuber count chamber according to procedure described by Fudge (2000) and Cray and Zaias (2004). Mean Corpuscular volume (MCV) was calculated by multiplying the percent hematocrit by ten divided by the erythrocyte count, Mean Corpuscular Haemoglobin (MCH) was calculated by the following equation: $(10 \times [Hb/RBC])$ and Mean corpuscular hemoglobin concentration (MCHC) was calculated by the following equation: $(100 \times [Hb/Hct])$. The activity of antioxidant enzymes in the plasma was analyzed using spectrophotometric assays. The ferric reducing ability of plasma (FRAP), which represents total antioxidant capacity, was determined according to Benzie and Strain (1996). The level of lipid hydroperoxides (LOOH) was determined according to (Gay and Gębicki, 2002). Catalase (CAT, EC 1.11.1.6), (SOD, EC 1.15.1.1), activity and levels of immunoglobulin A, M and G (IgA, IgM and IgG respectively), malondialdehyde (MDA), were determined by an immune-enzymatic ELISA assay using kits from Elabscience Biotechnology Co., Ltd. (Houston, TX, USA).

7.3. Serum biochemistry profile

Blood samples collected into plain bottles from the chickens were analyzed for serum through the colorimetry method using Jenway 6405 UV/VIS Spectrophotometer (UK). Serum protein was determined by the process described by Tietz and Norbert (1995). Bromo Cresol Green (BCG) method was used for serum albumin determination as described by Donmas, Watson and Briggs (1971). Alanine Transaminase (ALT), Aspartate Transaminase (AST) were determined using the process outlined by (IFCC 1986a; IFCC 1986b) and Cholesterol values recorded using the method described by Gordon and Amer (1977). Beam spectrophotometer (492 nm) was used to determine the Creatinine levels while the concentration of Low-Density Lipoprotein (LDL), High Density Lipoprotein (HDL) and Triglycerides in the serum were determined using the procedures described by Burtis and Ashwood (1999).

8. Statistical analysis

One-way analysis of variance was conducted using "STATISTICA 10 software" to evaluate significant differences between treatments and replicate means. Probability levels (P) which is equal to or less than 0.05 were considered significant in all tables. Results in tables are illustrated as means (X) ± Standard Deviation (SD). Note that all tables and figures represented in this manuscript illustrated the results achieved of both trials as one unit to show all resulting data of all experimental groups near each other to facilitate comparison and discussion, taking into consideration that sometimes the tables and figures as well might contain four control groups. It was necessary to clarify this in order not to be confused when comparing and discussing the results of each trial.

IV. RESULTS AND DISCUSSION

1. Health status and mortality

Daily observations showed no problems in chicks' health. The birds had good stature, very few leg weaknesses and showed no symptoms of diseases or feed deficiencies. Table 1 shows the variations in livability (percentage mortality) of birds during the experiment. Among the studied birds, a total of 55 mortalities occured referring to 7.83% mortaliy rate. The table show that EGVII (Chamomile), EGVIII (Rosemary + Chamomile), CGIb (Negative control of trial II) and CGIIb (positive control of trial II) showed the lowest mortality rate (1.82%) followed by EGV (Peppermint + Thyme) [3.64%], EGIII (Peppermint), EGIV (Thyme) and CGIa (Negative control of trial I) [5.45%], CGIIa (Positive control of trial II) [7.57%], EGVI (Rosemary) [10.91%], EGXI (Garlic + Onion) [12.73%], and EGIX (Onion) [14.55%]. However, the highest mortality rate was recorded in bird group EGX (Garlic) [25.45%], where the diet fed to the birds was supplemented with garlic powder.

Table 1. Summary table of health status and mortality (1-31 days)

Herbs	Live frequency	Live percentage	Dead frequency	Dead percentage
EG III (Peppermint)	51	7.88%	3	5.45%
EG IV (Thyme)	51	7.88%	3	5.45%
EG V (Peppermint + Thyme)	52	8.04%	2	3.64%
EG VI (Rosemary)	48	7.42%	6	10.91%
EG VII (Chamomile)	53	8.19%	1	1.82%
EG VIII (Rosemary + Chamomile)	53	8.19%	1	1.82%
EG IX (Onion)	46	7.11%	8	14.55%
EG X (Garlic)	40	6.18%	14	25.45%
EG XI (Garlic+Onion)	47	7.26%	7	12.73%
CG Ia (Negative)	51	7.88%	3	5.45%
CG IIa (Positive)	49	7.57%	5	9.09%
CG Ib (Negative)	53	8.19%	1	1.82%
CG IIb (Positive)	53	8.19%	1	1.82%
All Groups	647	92.17%	55	7.83%

2. Live body weight (LBW)

2.1. At 19 days of age

The average weight of one-day old chick was 42.02 ± 0.103g with no significant difference ($P>0.05$) among the thirteen experimental groups. The results obtained show no significant differences ($p>0.05$) among all experimental groups of both trials. Note that the highest was observed in group EG X- Garlic, where garlic was supplemented to the basic diet (555.02±28.66g) and the lowest mean values in two experimental groups EG VIII-Rosemary + Chamomile (531.44± 20.16g) and CG IIa- positive control of the 1st trial (531.8±22.18g) where a combination of antibiotics and antioxidants were added to the basic diet, respectively.

2.2. At 31 days of age

The obtained results show that broilers group EG VI- Rosemary fed with basal diet (1266.21 ± 52.09g) as well as the positive control of the first trial CGIIa (1251.66 ± 399.72g) were significantly ($P<0.05$) higher than groups EG IV (1116.41±63.82g) and EG VIII (1103±30.36g) fed with thyme and a combination of chamomile and rosemary, respectively. Moreover, a significant low level of LBW ($p<0.05$) was obtained between EG VIII- Rosemary + Chamomile (1103±30.36g) and EG III- Peppermint [1229.27±234.11g], EG V-Peppermint + Thyme (1215.89±140.53g), EG XI- Rosemary (1266.2±52.09g), EG IX- Onion (1225.61±126.33g). However, the highest numerical LBW at 31 days among the groups was recorded in EG VI- Rosemary (1266.2±52.09g) and the lowest was in EGVIII- Rosemary + Chamomile (1103±30.36g) [$P<0.05$].

3. Live body weight gain (LBWG)

The results show that average LBWG among all groups during the first period (19 days of age) no significant difference between the control group and the other groups. It is worthy to mention that after conducting the Bonferroni test we found that the highest LBWG ($P>0.05$) level was achieved in EG X- Garlic (500.02±28.66g) whose birds were fed a garlic supplemented basal diet and the lowest in EG VIII- Rosemary + Chamomile (476.44±20.16g) whose birds received basal diet supplemented with a combination of chamomile and rosemary *Vs* CG IIa- positive control (476.80±22.18g). LBWG among all groups during the finishing period (at 31 days of age) showed that birds fed with thyme (EG IV) as well as those fed with a combination of rosemary and chamomile (EG VIII) show the lowest LBWG among all the groups (571.83±64.70g and 571.84±40.22g, respectively) while birds whose diet was mixed with rosemary alone EG VI show the highest LBWG, 729.71±63.69g ($P<0.05$). EG VI- Rosemary was significantly ($p<0.05$) higher than EG IV- Thyme, EG VIII- Rosemary + Chamomile, EG XI- Garlic + Onion (606.47±155.35g), CG Ia- negative control for 1st trial (590.7±192.94g) and CG IIb- positive control for 2nd trial (584.23±131.74g). Moreover, CG IIa- positive control for 1st trial differed significantly from EG IV- Thyme, EG VIII- Rosemary + Chamomile and CG Ia- Negative control of the 1st trial ($P<0.05$). Figure 5 reveals the variation in LBWG during the whole experiment (1-31 days of age). EG VIII fed with a combination of rosemary and chamomile shows the lowest LBWG (1048.3±30.36g) among the experimental groups significantly differing ($P<0.05$) from EG III- Peppermint (1174.3±234.11g), EG V- Peppermint + Thyme (1160.9±140.53g), EG VI- Rosemary (1211.21±52.09g), EG IX- Onion (1170.61±126.33g) and CG IIa (1196.7±57.1g). The highest numerical results concerning LBWG were obtained in EG VI supplemented with rosemary and CG IIa where antibiotics and antioxidants were added to the basal diet. LBWG results of both groups were significantly higher ($p<0.05$) than EG IV (1061.41±8.94g), EG VIII (1048.3±4.2g), CG Ia (1073.1±26.8g). The highest numerical results concerning LBWG were obtained in EG VI supplemented with rosemary and CG IIa where antibiotics and antioxidants were added to the basal diet. LBWG results of both groups were significantly higher ($p<0.05$) than EG IV (1061.41±63.82g), EG VIII (1048.3±30.36g), CG Ia- Negative control of 1st trial (1073.1±191.25g).

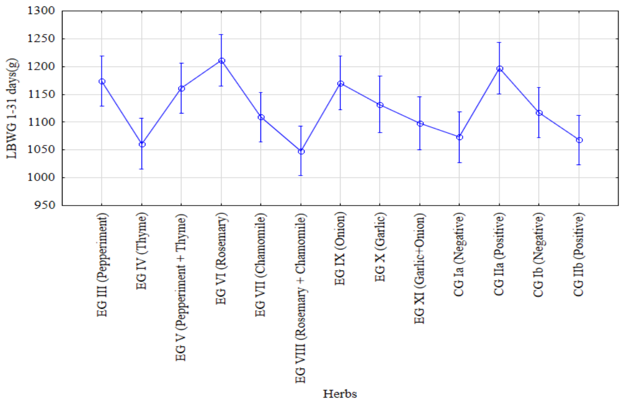

Figure 5. Variations in average live body weight gain during the whole experimental period (1-31 days of age) among groups, g

4. Feeding and FCR

During the starter period, the highest feed intake was noticed in both EG V- peppermint + Thyme (38.17kg/group) and EG VIII- Rosemary + Chamomile (38.16kg/group) where the birds were fed with a combination of peppermint and thyme, chamomile, and rosemary respectively. However, the lowest FI was recorded in EG X- Garlic (32.89kg/ group) where garlic powder was added to the basal diet Vs all groups especially EG IX- Onion (35.27 kg/group), CG Ib- negative control of the 2nd trial (36.74 kg/group) and CG IIb- positive control of the 2nd trial (33.58 kg/group). When it comes to the calculated FCR during the first period of the trials, a low of 1.28 was obtained in group CG IIb where antibiotics and antioxidants were mixed with the diet fed to the birds. The inferior apparent value of FCR among treated groups in ascending order was observed in EGVI (1.60) where a supplementaion of rosemary was added to the basal diet. FI and FCR during the grower phase (20-31 days of age) were measured and calculated respectively. During the grower phase (20-31 days of age), feed consumption was increased in the experimental groups. The highest feed intake remained at the level of EG V- Peppermint + Thyme (56.73kg/group) while the lowest was seen in EG III- Peppermint (41.60 kg/group). Increased feed consumption may be attributed to flavoring effects of combining peppermint with thyme. The inferior rate of FCR was observed in EG VII- Chamomile (1.87) where chamomile was added to basal diet in comparison with other groups whereas the most effective FCR was that of EG VI- Rosemary (1.08) where the fed diet was supplemented with rosemary. Results of FI as well as the apparent FCR at slaughter from day 1 to 31 days are shown in table 2. Once more data shows that combining peppermint with thyme (EG V) and adding them to the basal diets fed to birds show the highest FI (94.90kg/group). A high result (92.29kg/group) was also obtained when the birds received chamomile (EG VII). Moreover, birds of groups EG III- Peppermint, EG IV- Thyme and EG X- Garlic showed the lowest values of FI (77.74, 77.87 and 77.89 kg/group, respectively).Feed conversion was inferior in EG X- Garlic (1.72) and EG XI (Garlic + Onion (1.68) in comparison to all groups where birds of the first received a garlic supplementation whereas that of the second were offered a combination of garlic and onion powders. Adding to what was mentionned, the most effective and superior FCR values was achieved in EG IV- Thyme (1.29) and EG VI- Rosemary (1.3) whose diets were supplemented with thyme and rosemary, respectively.

Table 2. FI and FCR at 1-31days of age

Parameter / Group	Feed intake (1-31 days of age, kg)	Feed conversion ratio (1-31 days of age)
EGIII (pepper-mint)	77.74	1.36
EGIV (thyme)	77.87	1.29
EGV (peppermint + thyme)	94.90	1.59
EGVI (rosemary)	83.02	1.30
EGVII (chamo-mile)	92.29	1.63
EGVIII (rosemary + chamomile)	88.21	1.53
EGIX (onion)	80.92	1.50
EGX (garlic)	77.89	1.72
EGXI (garlic + onion)	86.92	1.68
CGIa (negative)	88.49	1.54
CGIIa (positive)	82.89	1.51
CGIb (negative)	88.35	1.49
CGIIb (positive)	79.69	1.41

5. Carcass Yield

At slaughter the feather was manually plucked, legs removed, and the carcass was eviscerated where all internal visceral organs were eradicated, leaving the liver and heart intact for further weighing. Data shows no significant (p>0.05) difference in the percentages of NW in relation to LBW at slaughter among all groups except EGVIII where the birds fed with a basal diet supplemented with a combination of chamomile and rosemary shows a significant difference ($p<0.05$) when compared to other groups with a percentage of 63.77±15.39%. Best numerical NW, % was achieved in CGIIa (76.03 ± 1.87%) where antibiotics and antioxidants were added to the diet followed by the other experimental groups that all showed a result higher than 71%.

6. Weight of liver+heart (edible organs)

The percentages of edible organs (liver, heart) to the net weight of the bird ranged between 3.1±0.41% for EG VI- rosemary and 5.33±0.36% for EG XI-garlic + onion. Compared to the other experimental groups, birds belonging to EG XI fed with a diet supplemented with a mixture of garlic and onion showed the highest numerical percentage (5.33±0.36%) significantly different ($p<0.05$) from EG IV (3.53±0.21%), EG V (3.4±0.42%), EG VI (3.1±0.41%), EG VII (3.64±0.07%), EG VIII (3.97±0.19%), CG Ia (3.87±0.27%) and CG IIa (3.9±0.04%). When it comes to the lowest percentage (3.1±0.41%) concerning EG VI, it was significantly different from the percentages of EG IX (4.52±0.14%), EG X (4.65±0.19%), EG XI (5.33±0.36%), CG Ib (4.59±0.22%) and CG IIb (4.62±0.22%).

7. Breasts (left+right) % to NW

The results obtained show a significant difference ($p<0.05$) between EG IX (26.82±1.73%) whose birds were fed with a basal diet supplemented with onion compared to the other groups except EG X (24.14±1.55%) and EG XI (23.24±0.87%) showing results that are not considered to be significantly different from EG IX ($P>0.05$). However, birds belonging to EG III where peppermint was used as a feed additive show the lowest breast yield to net weight percentage (20.63±0.8%).

Meat quality

8. Meat pH

8.1. After 24h of cooling

After 24h of cooling the registered meat pH for all the experimental groups is shown in figure 6.

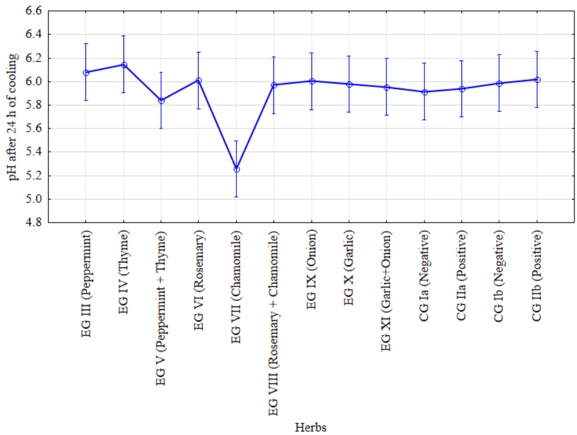

Figure 6. pH variation after 24h of cooling

From the figure above, we can notice that the pH numerically differed slightly between the groups. However, the remarkable significant difference ($p<0.05$) was recorded at the level of EG VII whose birds were fed a basal diet supplemented with chamomile with pH (5.26±0.16) being the lowest among all groups. Moreover, we can note that EG IV had the highest numerical pH value (6.14±0.09) with no significant difference as compared to the rest of groups except when compared to EG VII.

8.2. After 1 month of freezing

After 1 month of freezing, the pH was measured to track its evolution and to check any potential effect of the studied herbs on the pH values. The obtained results are exposed in figure 7.

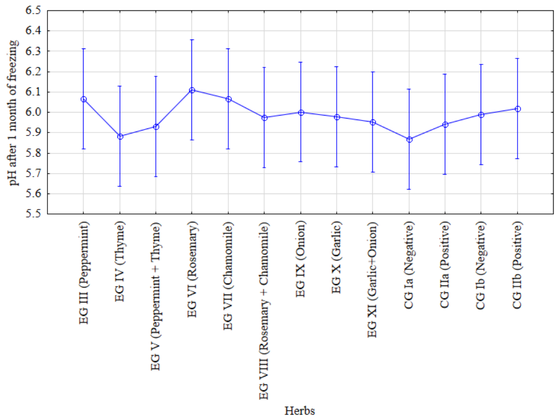

Figure 7. pH variation after 1 month of freezing

In the present experiment, results in figure 7 show that there is no significant difference between the groups according to pH after 1 month of freezing. The obtained values ranged between 5.86±0.03 for CG Ia fed with the basal diet without antibiotics and antioxidants and 6.11±0.22 for EG VI where the birds were fed with a diet mixed with rosemary used as feed additive.

9. Trichromatic coordinates

9.1. Lightness (L*)

9.1.1. After 24h of cooling

No significant differences were noticed among the obtained results except only at the level of one result. L* of EG VIII (51.13±2.76) whose birds were fed with a basal diet supplemented with a combination rosemary and chamomile was significantly lower ($p<0.05$) than the lightness of EG VI where rosemary was added only to the basal diet of the birds (58.43±1.9).

9.1.2. After 1 month of freezing

Figure 8 shows the lightness (L*) values corresponding to the experimental groups after 1 month of freezing.

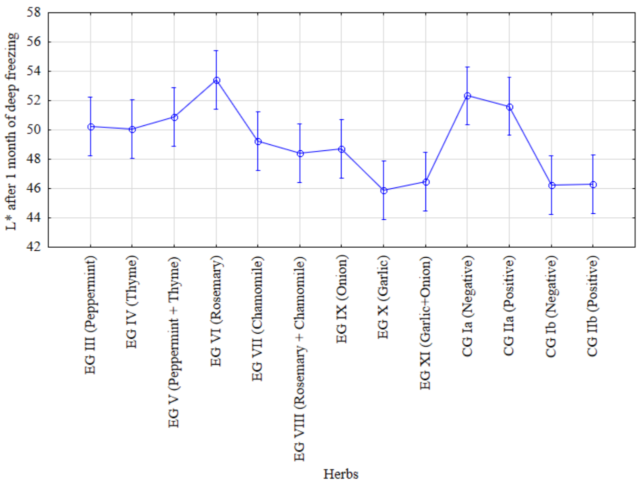

Figure 8. Lightness (L*) after 1 month of freezing

The figure above clearly shows many significant differences with many of the studied groups. EG VI, referring to the birds whose diet was supplemented with rosemary showed s a high L* value (53.43±1.73) significantly differing ($p<0.05$) from EG X (45.9±0.43), EG XI (46.46±0.58), CG Ib (46.24±0.66) and CG IIb (46.32±0.57). Moreover, EG X where the used feed additive was garlic as well as EG XI where the diet fed to birds was supplemented with a mixture of garlic and onion powders showed a significantly ($p<0.05$) lower L* values (4.59±0. 43; 46.46±0.58) respectively as compared to EG VI (53.43±1.73), CG Ia (52.33±0.07) and CG IIa (51.62±0.17). In addition, CG Ia (52.33±0.07) and CG IIa showed higher significantly different ($p<0.05$) L* values than CG Ib (46.24±0.66) and CG IIb (46.32±0.57).

9.2. Redness (a*)

9.2.1. After 24h of cooling

Obtained results of a* measured at 24 hours *postmortem* did not differ significantly ($p>0.05$) among all the treatments compared with the control group. However, the highest redness value was obtained in the positive control group CG IIa (8.33±0.1) where the birds were fed with the basal diet in addition to antibiotics and antioxidants. The lowest a* values were spotted in EG VII (6.39±0.5) and EG VIII (6.39±0.2) to which chamomile and a combination of chamomile and rosemary were added respectively.

9.2.2. After 1 month of freezing

Redness not differing significantly ($p>0.05$) after 24 h of cooling among the experimental groups showed some significant differences ($p<0.05$) after 1 month of freezing (fig. 9). Birds fed with a basal diet supplemented with onion (EG IX) showed a significantly ($p<0.05$) higher a* value (9.91±0.91) than EG X (6.57±052), CG Ib (7.25±0.68) and CG IIb (7.1±0.73). Moreover, a* value obtained for EG X where birds were fed with a basal diet mixed with garlic was significantly ($p<0.05$) lower than the results of EG IX (9.91±0.91), CG Ia (9.37±0.19 and CG IIa (10.27±0.31). However, the highest numerical redness value was that of CG IIa.

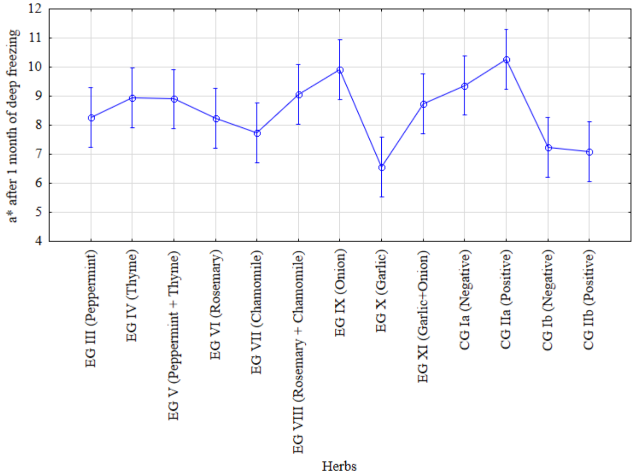

Figure 9. Redness (a*) after 1month of freezing

9.3. Yellowness (b*)

9.3.1. After 24h of cooling

Birds belonging to EG IX (8.95±0.51), EG X (7.58±0.7), EG XI (8.31±0.56), CG

Ib (8.41±0.51) and CG IIb (8.45±0.42) all showed significant differences (p<0.05) when compared to EG III (12.9±0.24), EG IV (12.1±0.08), EG V (12.41±0.19), EG VI (12.84±0.03), EG VII (13.62±0.06), EG VIII (12.07±0.06), CG Ia (13.54±0.22) and CG IIa (11.57±0.65).

9.3.2. After 1 month of freezing

Samples frozen for 1 month significantly differed (p<0.05) within each other (fig. 10). Birds fed with a combination of peppermint and thyme in addition to the basal diet (EG V) showed a significantly (p<0.05) higher b* value (13.95±0.15) than EG VIII (10.93±0.68), EG IX (10.87±0.45), EG X (8.29±0.55), EG XI (9.27±0.59), CG Ib (9.01±0.34) and CG IIb (9.00±0.44). Moreover, yellowness of EG X (8.29±0.55), EG XI (9.27±0.59), CG Ib (9.01±0.34) and CG IIb (9.00±0.44) were significantly (p<0.05) lower than b* of EG III (12.7±0/06), EG IV (12.58±0/32), EG V (13.95±0.15), EG VI (12.91±1.11) and EG VII (12.88±1.27).

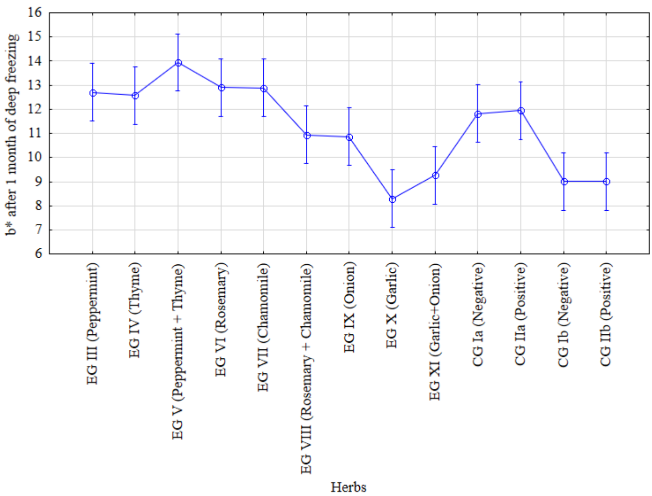

Figure 10. Yellowness (b*) after 1 month of freezing

The results achieved on broiler breast color during the experiment. As was expected L* color after freezing was darker in all groups whereas control group CG Ia had the lighter numerical value 52.4 in comparison with all other treatments CG IIa (51.4), EG III (50.9), EG IV (52), EG V and EG VI (51.8), EG VII (51.5) and EG VIII (50.9). Redness a* was higher in all groups after 7 days of deep freezing and Yellowness b* of meat attained almost the same level as after 24 hours *post-mortem*. This is in contradiction with the findings of Barbut (1997) who found that L* (lightness) value was significantly higher in the cinnamon powder groups than the control group, but there was no significant difference in a * (redness) and b* (yellowness) values among groups. The meat color is a major criterion that is used by consumers to judge meat quality. The L* value is especially important in white muscles and is correlated with drip loss and pH. These results coincide with those achieved by Nasir (2009) who reported that effects of supplementation of *Echinacea purpurea*, *Nigella sativa* and their combined application on meat color of broilers is lower in L* than control group. Color (Myoglobin Proteins) Myoglobin has been identified in exudates by gel electrophoresis, accounting in part for the change in the color stability of meat after freezing and thawing (Anon and Cavelo, 1980). It has also been reported that denaturation of the globin moiety of the Myoglobin molecule takes place at some stage during freezing, frozen storage and thawing (Ambrosiadis *et al.* 1991). The denaturation leads to an increased susceptibility of Myoglobin to autoxidation and subsequent loss of optimum color presentation. This theory has been verified by many authors by comparing the degree of bloom and the ability of the meat to resist oxidation to metmyoglobin during refrigerated storage post freeze/thaw (Marriott, *et al.*, 1980; Lanari and Zaritzky, 1991; Abdallah *et al.*, 1999; Otremba *et al.*, 1999). The existence of an enzyme system capable of reducing metmyoglobin back to Myoglobin was proposed by Livingston and Brown (1981) and was termed the metmyoglobin reducing activity (MRA). The theory is that in fresh muscle the enzyme is very active and the metmyoglobin formed is quickly reduced to deoxymyoglobin and oxygenated back to oxymyoglobin, thereby retaining the bloomed color. However, as the meat ages or is frozen, the activity of the MRA is decreased and metmyoglobin begins to accumulate on the surface of the meat at a rapid rate (Abdallah *et al.*, 1999). Also, MRA and/or co-factors, such as NADH, could be 'lost' from the post mortem sarcoplasmic environment by leaching as exudates during thawing, and/or due to oxidation, and/or be used by reactions unrelated to MRA, which will all contribute to accelerated oxidation and loss of bloom (Abdallah *et al.*, 1999). Kirkpinar *et al.* (2014) found that dietary oregano and garlic oil supplementation did not affect carcass yields, the relative weight of carcass parts, breast and thigh meat composition, pH or *b** value of breast meat. Oregano + garlic oil supplementation significantly decreased the *L** value. The *a** value of breast meat in birds given a diet supplemented with oregano oil was lower than that in birds given a diet supplemented with garlic oil and oregano oil + garlic oil. The essential oil addition had no positive effect on the oxidative stability. There was no difference between the treatments in breast appearance.

10. Drip loss after 24h

The drip loss measured 24h after cooling gave the results figuring out below (fig. 11). Birds belonging to EG IV were fed with a basal diet supplemented with thyme. It is obvious that drip loss of that group was significantly ($p<0.05$) lower (1.6±0.02%) than EG III (3.17±0.04%), EG V (3.24±0.05%), EG VI (2.94±0.12%), EG VII (3.4±0.05%), CG Ia (2.63±0.07%) and CG IIa (3.16±0.07%). EG VII whose birds received chamomile as feed additive showed a significantly($p<0.05$) higher (3.4±0.05%) yellowness than EG IV (1.6±0.02%),

EG VIII (2.28±0.13%), EG IX (2.37±0.3%), EG X (2.3±0.47%), EG XI (1.8±0.2%), CG Ib (2.15±0.17%) and CG IIb (2.1±0.26%). Supplementing the basal diet with a combination of garlic and onion (EG XI) powders seemed to result in a significantly (p<0.05) lower b* values (1.8±0.2%) than EG III (3.17±0.04%), EG V (3.24±0.05%), EG VI (2.94±0.12%), EG VII (3.4±0.05%) and CG IIa (3.16±0.07%).

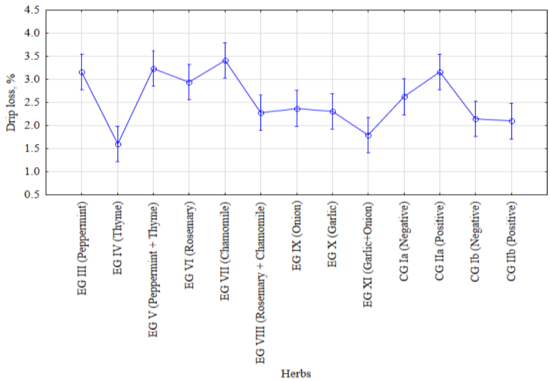

Figure 11. Drip loss after 24h of cooling (%)

Our result was in contradiction to the findings of Begum (2014) who postulated that drip loss did not differ among treatment groups fed with different types of herbs. This can be explained in agreement with Santos *et al.* (2004) who illustrated that fresh meat from slaughtered animals contains about 70% water which is essential to its quality, but it begins to leak away soon after death. Moreover, boning and cutting can result in losses of 1-2% and further long-term storage can lead to much greater losses of up to 12%. They added that drip loss on this scale represents a large reduction in the yield of meat leading to financial losses as well as affecting the appearance, nutritional value and palatability of the meat to the consumer.

11. Thawing loss after 1 month of freezing

Thawing loss assessed after 1 month of freezing revealed significant differences (P<0.05) among the studied groups (fig. 12). Results obtained for EG IX (8.7±0.96%), EG X (5.4±0.84%), EG XI (10.25±1.4%), CG Ib (6.75±1.23%) and CG IIb (5.14±0.74%) were significantly (p<0.05) lower than thawing loss values of EG III (14.9±0.37%), EG IV (14.13±0.23%), EG V (17.71±0.23%), EG VI (14.21±0.13%), EG VII (18.63±0.33%), EG VIII (14.67±0.22%), CG Ia (15.6±0.13%) and CG IIa (17.11±0.2%). However, the highest numerical value of thawing loss percentage was obtained for EG VII where the diet fed to the birds was supplemented with chamomile (18.63±0.33%), whereas the lowest was that of CG IIb (5.14±0.74%) where antibiotics and antioxidants were supplemented to the basal diet. This could be explained by the conservative properties of Rosemary and Thyme resulting in better broiler meat quality as reported by Hengl (2011).

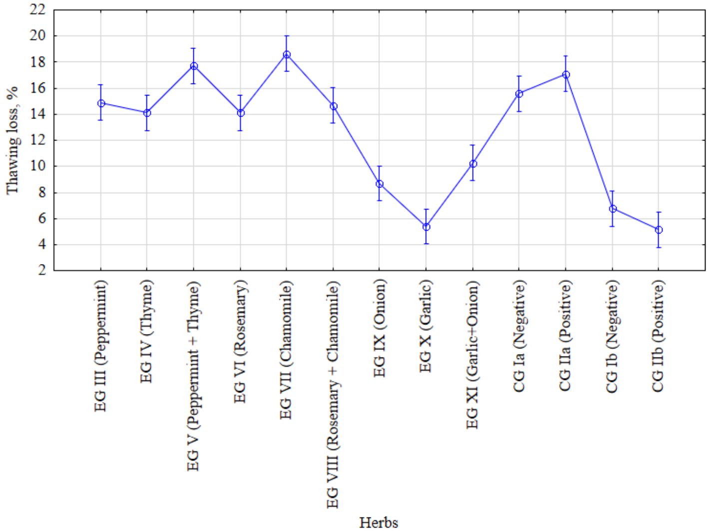

Figure 12. Thawing loss after 1 month of freezing (%)

12. Cooking Loss

12.1. After 24h of cooling

Figure 13 shows the variation of cooking loss percentage as a function of the studied herbs after 24h of cooling. Cooking loss percentages for EG IX (7.78±0.52%) and EG X (10.48±0.72%) were significantly ($p<0.05$) lower than EG III (18.67±1.64%), EG IV (17.73±2.26%), EG V (19.2±0.22%), EG VI (18.03±0.34%), EG VII (20.37±1.55%), EG VIII (18.67±2.56%), CG Ia (17.67±1.1%) and CG IIa (21.77±0.96%). Moreover, birds fed with a diet supplemented with a combination of garlic and onion showed a significantly ($p<0.05$) lower cooking loss (11.9±0.8%) percentage than EG III (18.67±1.64%), EG V (19.2±0.22%), EG VII (20.37±1.55%), EG VIII (18.67±2.56%) and CG IIa (21.77±0.96%) where the diets fed to the birds were mixed with peppermint, combination of peppermint and thyme, chamomile, a mixture of chamomile and rosemary and antibiotics respectively. However, the highest numerical percentage was observed in CG IIa (21.77±0.96%) supplemented with antibiotics and the lowest in EG IX (7.78±0.52%) to which onion was added.

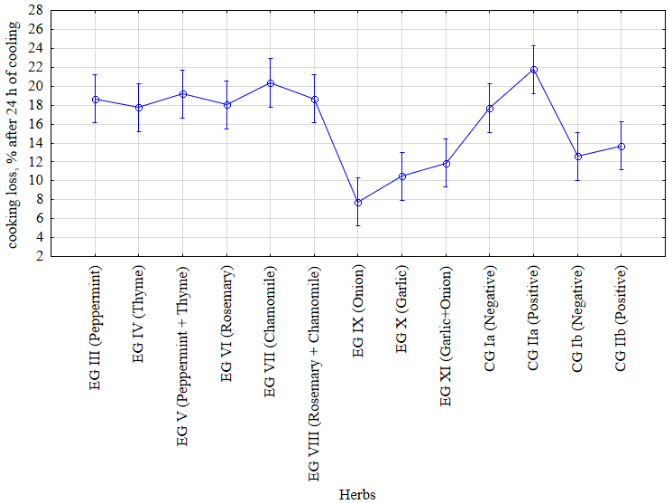

Figure 13. Cooking loss after 24h of cooling (%)

12.2. After 1 month of freezing

After 1 month of freezing, cooking loss percentage results are shown in figure 14 below. The obtained values showed significant differences ($p<0.05$) between the experimental groups. It is obvious that birds that where fed with a diet supplemented with onion (EG IX) showed the lowest cooking loss percentage (13.35±1.09%) significantly ($p<0.05$) less than the percentages of EG III (23.72±2.71%), EG IV (26.96±3.11%), EG V (28.75±0.31%), EG VI (26.25±0.49%), EG VII (28.72±0.13%), CG Ia (26.37±1.87%) and CG IIa (29.76±1.65%). Moreover, when garlic added to basal diets (EG X) as well as CG Ib and CG IIb cooking loss percentages were also significantly ($p<0.05$) lower (16.09±0.73%; 16.93±1.16% and 16.63±0.9% respectively) than EG IV, EGV, EG VI, EG VII, CG Ia and CG IIa.

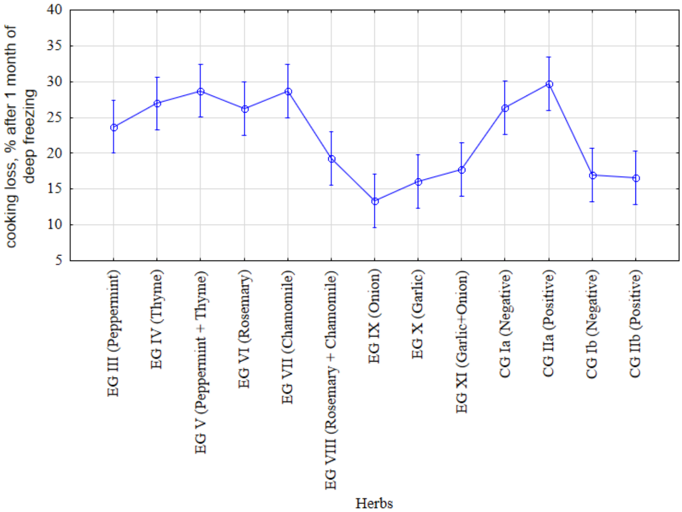

Figure 14. Cooking loss after 1 month of freezing (%)

Cooking yield was observed to be higher in breasts of broiler fed 1% mix of Rosemary and Chamomile meals when compared with other types of supplementations however, the difference was not significant (P>0.05). Thyme and Rosemary meal inclusion in broiler diet had no significant effect (P>0.05) on cooking loss of broiler meat even though the results obtained were numerically lower. This result of cooking yield is similar to that of Fayeye (1994) and Ayoola (2014) who reported that cooking yield increased with the level of mango seed kernel meal in rabbit diets however, it was not statistically (P>0.05) significant.

13. Meat tenderness

13.1. After 24h of cooling

Results of the present study show that the tenderness of breast meat did not differ significantly between birds fed different diets after 24 h of cooling. EG X whose birds were fed with a basal diet supplemented with garlic was the lowest 6.52±0.33 mm. However, chamomile supplementation showed the highest meat tenderness among the studied groups (8±0.54mm).

13.2. After 1 month of freezing

Meat tenderness after 1 month of freezing also showed no significant differences among different experimental groups. Combining peppermint and thyme and adding them to the diet (EG V) gave the highest meat tenderness (7.01±0.29mm) while the lowest was obtained in CG Ib (5.78±0.3mm) where the birds was fed with the basal diet without any additives or antibiotics.

14. Serum biochemistry of broiler chickens fed different herbs and hematological indices

Table 3 shows the effect of supplemented diets on the hematological indices of broiler chickens at slaughter. There was no significant difference ($p>0.05$) in the RBC, mean corpuscular volume, and mean corpuscular hemoglobin concentration of chickens fed the experimental diets. However, PCV and hemoglobin count were increased ($p<0.05$) in chickens group fed basal diet + chamomile and chamomile + rosemary. WBC increased ($p<0.05$) in chickens group fed basal diet + Rosemary and chamomile. Heterophils was reduced ($p<0.05$) in chickens groups fed basal diet + peppermint + thyme and basal diet + rosemary. Lymphocyte was increased ($p<0.05$) in the group of chickens fed basal diet + rosemary than chickens on basal diet only. Also, the eosinophils increased in chickens on basal diet + rosemary and chamomile. Also, the group of chickens fed basal diet + peppermint + thyme and basal diet + rosemary + chamomile had increased ($p<0.05$) basophils counts. Furthermore, chickens group fed basal diet + rosemary had increased ($p<0.05$) monocyte count, followed by chickens group fed basal diet + rosemary + thyme and basal diet + chamomile. Chickens on Basal diet + rosemary had increased ($p<0.05$) mean corpuscular hemoglobin count than chickens fed basal diet only. Providing the diets with herbal based growth promoters to the diet had significant effects on some blood parameters compared to diets with no supplementations in specific in the case where a mixture of chamomile and rosemary was added to the diet. It has been reported previously by Pournazari et al., (2017) that the dietary inclusion of thyme and other phytobiotics improved the blood serum and lipid parameters which coincides with our findings (Attia et al., 2017).

Table 3. Effect of supplemented diets at slaughter on Hematological Indices of broiler chickens.

Parameters	Haemoglobin(g/dl)	RBC (× 10^{12}/L)	WBC (× 10^{9}/L)	Heterophils (%)	Lymphocyte (%)	Eosinophils (%)	Basophils (%)	Monocytes (%)	PCV (%)	Mean corpuscular volume (fl)	Mean corpuscular haemoglobin (pg)	Mean corpuscular haemoglobin concentration (g/dl)
EG III (Peppermint)	11.450^{a}	2.950a	10.275^{a}	39.000^{a}	61.250^{a}	0.000^{a}	0.250^{a}	0.250^{a}	33.000^{a}	110.750a	36.922^{a}	32.850a
EG IV (Thyme)	11.400^{a}	2.750a	11.650^{a}	35.500^{a}	63.500^{a}	0.250^{a}	0.250^{a}	0.000^{a}	33.000^{a}	115.800a	38.947^{a}	31.800a
EG V (Peppermint + Thyme)	9.825^{a}	2.550a	11.850^{b}	28.550b	69.250^{b}	0.250^{a}	0.750^{b}	0.500^{a}	32.500^{a}	112.275a	37.887^{a}	33.452a
EG VI (Rosemary)	11.575^{a}	2.700a	9.975^{a}	29.550^{b}	70.500^{b}	0.250^{a}	0.000^{a}	1.000^{b}	36.000^{a}	117.435a	40.892^{b}	33.320a
EG VII (Chamomile)	12.475^{b}	3.200a	10.700^{a}	33.500^{a}	64.000^{a}	0.250^{a}	0.250^{b}	0.500^{a}	37.000^{b}	116.780a	39.022^{a}	32.912a
EG VIII (Rosemary + Chamomile)	12.987b	2.950a	12.150b	34.250a	64.550a	0.750b	0.750b	0.000a	39.500b	118.650a	35.850a	34.350a
EG IX (Onion)	11.580a	2.850a	10.550a	35.850a	64.000a	0.000a	0.000a	0.250a	36.000a	115.300a	36.865a	33.720a
EG X (Garlic)	9.580a	2.950a	9.950a	35.800a	36.500a	0.250a	0.000a	0.250a	36.500a	115.850a	35.840a	33.625a
EG XI (Garlic+Onion)	10.854a	2.700a	9.500a	34.850a	36.550a	0.250a	0.250a	0.250a	35.000a	116.500a	36.050a	34.950a
CG Ia (Negative)	11.650a	2.650a	10.650a	33.500a	60.250a	0.250a	0.250a	0.000a	35.000a	112.350a	36.500a	31.600a
CG IIa (Positive)	11.879a	2.650a	10.840a	33.800a	59.250a	0.250a	0.000a	0.250a	34.500a	113.600a	35.890a	31.400a
CG Ib (Negative)	11.587a	2.650a	10.760a	32.850a	61.550a	0.250a	0.250a	0.250a	35.500a	111.600a	35.780a	31.900a
CG IIb (Positive)	11.698a	2.750a	11.000a	31.550a	60.550a	0.250a	0.000a	0.000a	35.000a	112.800a	35.650a	32.500a
SEM	0.316	0.093	0.236	1.201	1.135	0.081	0.093	0.104	0.941	1.605	0.537	0.264

SEM = Standard Error of Mean.
g/dl = gram/decilitre.
fl = femtolitre.
pg = pictograms.
[ab]Means in the same column with different superscripts differ significantly ($p<0.05$).

The results of the analysis showed that dietary treatments significantly ($p<0.05$) influenced most of the hematological parameters studied. A mixture of rosemary + chamomile had the highest value of packed cell volume (39.5%), hemoglobin (12.88 g/dl) and white blood cell (WBC) count (12.15×109L-1). Packed cell volume (PCV) is the percentage of Red Blood cells (RBC) in blood (Purves et al., 2003). Moreover, haemoglobin forms about one third of the blood. Some herbal extracts containing phenol can inhibit iron absorption and decrease haemoglobin levels, which in our case was not reported where an increase was observed when adding a mixture of rosemary and chamomile. Adaptation of chickens to antioxidants fed, as well as proper absorption of iron from feed at the finisher phase may explain high values for circulating red blood cells in treatment group mentioned above. Moreover, in this group total protein and albumin were significantly higher ($p<0.05$). In addition, HDL, and LDL values were better in birds supplemented with rosemary + chamomile compared to other groups. It can be concluded that rosemary + chamomile at 5 :5g/kg improved PCV, Hb, HDL and LDL concentrations in broiler chicken. In different studies conducted to evaluate the effect of essential oils (EO) on serum Tch, LDL and HDL levels contradictory results have been obtained. The increased in cholesterol concentration in thyme group could be due to the phenolic compounds of carvacrol and thymol that exhibit considerable antimicrobial activity (Vasudeva and Sharma, 2012) that may depress fat absorption due to bile acid deconjugation (El-Khoury et al., 2017). Several herbs and EO have been reported as having immunomodulatory effects such as lymphocyte expression, phagocytosis, modulation of cytokine and immunoglobulin secretion, histamine release and so on (Chowdhury et al., 2018). It has been reported plant extracts have positive immune effects such as increase in lymphocyte proliferation rate, phagocytic rate as well as increase in immunoglobulins such as IgA and IgM in the blood of broiler chickens (Reis et al., 2018). It has also been documented that EO effectively reduces the pro-inflammatory cytokine production and thereby attenuates the 2,4,6-trinitrobenzenesulfonic acid-induced colitis in mice (Manafi et al., 2014). Tables 4 and 5 show the effect of feeding diets supplemented with different herbs on the serum traits of broiler chickens at slaughter. At the finisher phase, total serum protein and albumin were higher in chickens fed basal diet + 1% Rosemary and 1% Chamomile flowers meals, onion and mix of onion and Garlic than chickens fed basal diet + other herbal supplement. Globulin levels were significantly ($p<0.05$) higher in chickens fed basal diet + Chamomile than chickens fed basal diet only and basal diet + all other supplements. However, globulin level was highest ($p<0.05$) in group of chickens fed Basal diet + chamomile and rosemary supplement amongst all groups while ALT was lower in the chickens fed basal diet + all other supplements and control groups except in the group fed with a mixture of rosemary and chamomile. There was no significant (P>0.05) variation in the AST and Creatinine of chickens fed the experimental diets. However, total cholesterol was lower, and HDL were higher (p<0.05) in chickens group fed basal diet + Chamomile and rosemary supplement compared to chickens group fed other experimental diets. Contrast analysis showed that the levels of LOOH in the chickens from all groups receiving the experimental supplementations were lower (p < 0.05) than in control groups G-C (table 9). Meanwhile MDA in rosemary, chamomile and their mixture (rosemary + chamomile) were significantly (p<0.05) lower than all other groups. Moreover, Catalase activity in the chickens from the same treatments mentioned was higher than all experimental groups (P< 0.05) but almost equal values to control groups (P>0.05). Besides, SOD values in all experimental groups were insignificant (P>0.05) relating to control groups. Zhu et al., (2014) and Rahimi et al., (2011) reported that the dietary inclusion of herbals extracts significantly increased the levels of total proteins and albumins which coincides with our findings where adding a mixture of rosemary and chamomile increased the total proteins and albumin content in the serum. Higher concentrations of total serum protein, albumin or globulin can be associated with increased nutrient intake. Ghazvinian et al., (2018) reported that the addition of different compounds of medicinal herbs reduce the activity of reductase enzyme which is the key enzyme in cholesterol synthesis, therefore lower cholesterol, LDL and VLDL are obtained. In our case, HDL was higher when birds were supplemented with a mixture of rosemary and chamomile or a mixture of onion and garlic powders. Moreover, LDL concentration decreased in the same groups which is beneficial for the birds.

Table 4. Effect of supplemented diets on Blood Serum and Lipid parameters of broiler chickens.

Parameters	Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Creatinine (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Total Cholesterol (mg/dl)
EG III (Peppermint)	3.006[a]	2.345[a]	1.484[a]	1.109a	115.120[a]	82.800[a]	30.100[a]
EG IV (Thyme)	3.203[a]	1.744[a]	1.253[a]	1.100[a]	90.685[a]	43.187[b]	29.175[a]
EG V (Peppermint + Thyme)	3.465[a]	1.987[a]	1.591[a]	1.155[a]	120.589[a]	73.104[a]	26.950[a]
EG VI (Rosemary)	3.990[a]	2.200[a]	1.890[a]	1.175[a]	97.385[a]	50.875[b]	25.305[b]
EG VII (Chamomile)	4.375[a]	2.575[a]	2.820[b]	1.285[a]	120.100[a]	73.870[a]	25.375[b]
EG VIII (Rosemary + Chamomile)	4.980[b]	2.750[b]	2.154[a]	1.247[a]	125.582[b]	42.189[b]	24.258[b]
EG IX (Onion)	4.587[b]	2.872[b]	1.758[a]	1.785[a]	121.015[a]	41.563[b]	24.235[b]
EG X (Garlic)	4.054[a]	1.987[a]	1.889[a]	1.145[a]	121.365[a]	41.879[b]	23.258[b]

EG XI (Garlic+Onion)	4.689^{b}	1.897^{a}	1.325^{a}	1.098^{a}	124.987^{b}	42.123^{b}	22.560^{b}
CG Ia (Negative)	3.220^{a}	0.997^{a}	1.452^{a}	1.089^{a}	99.358^{a}	81.370^{a}	25.221^{b}
CG IIa (Positive)	3.195^{a}	1.258^{a}	1.627^{a}	1.105^{a}	101.256^{a}	80.258^{a}	25.128^{b}
CG Ib (Negative)	2.983^{a}	1.658^{a}	1.325^{a}	1.087^{a}	105.237^{a}	79.347^{a}	24.987^{b}
CG IIb (Positive)	3.018^{a}	1.897^{a}	1.325^{a}	1.201^{a}	101.859^{a}	81.231^{a}	26.365^{b}
SEM	0.168	0.125	0.101	0.094	6.008	3.978	1.337

SEM = Standard Error of Mean.
HDL = High Density Lipoprotein.
LDL = Low Density Lipoproteinre.
g/dl = gram/decilitre.
mg/dl = milligram/decilitre.
^{ab}Means in the same column with different superscripts differ significantly (p<0.05).

Table 5. Effect of supplemented diets on Blood enzymes.

Parameters	ALT (U/L)	AST (U/L)	LOOH µmol/L	MDA µmol/L	SOD U/mL	CAT U/mL
EG III (Peppermint)	26.100^{a}	62.800^{a}	3.69a	0.65a	29.94a	2.60a
EG IV (Thyme)	26.4120^{a}	60.000^{a}	2.43a	0.54a	31.10a	2.64 a
EG V (Peppermint + Thyme)	27.498^{a}	62.000^{b}	2.09a	0.53a	29.01a	2.61a
EG VI (Rosemary)	25.250^{a}	66.500^{a}	3.80a	0.44b	31.46a	1.49b
EG VII (Chamomile)	28.150^{a}	58.800^{a}	3.89a	0.35b	29.89a	1.39b
EG VIII (Rosemary + Chamomile)	32.547^{b}	64.500^{a}	2.60a	0.33b	29.94a	1.29b
EG IX (Onion)	25.564^{a}	68.500^{a}	3.70a	0.64a	31.05a	2.68a
EG X (Garlic)	25.879^{a}	67.900^{a}	3.69a	0.65a	32.54a	2.57a
EG XI (Garlic+Onion)	25.874^{a}	62.300^{a}	3.70a	0.66a	33.08a	2.54a
CG Ia (Negative)	24.325^{a}	64.500^{a}	5.01b	0.65a	32.09a	1.94b
CG IIa (Positive)	25.365^{a}	65.800^{a}	4.98b	0.59a	32.12a	1.87b
CG Ib (Negative)	25.325^{a}	64.000^{a}	4.92b	0.64a	33.01a	1.91b
CG IIb (Positive)	24.958^{a}	66.000^{a}	4.97b	0.64a	32.25a	1.94b
SEM	0.802	2.090	0.101	0.035	0.46	0.09

SEM = Standard Error of Mean.
ALT = Alanine aminotransferase.

AST = Aspartate aminotransferase.
U/*L*= Unit/Litre
μmol/L= micromole/Litre

As shown in the table 5 above, no effect was recorded at the level of AST and SOD, while ALT increased in the group whose birds were supplemented with a mixture of rosemary and chamomile which could be due to higher nutrient intake and coincides with the findings of Senanyake et al., (2015). ALT is responsible of converting alanine to pyruvate, for better cellular energy production. A mixture of rosemary and chamomile in the diet would promote healthier chicks. Moreover, experimental groups showed lower LOOH values compared to control groups. Adding to that, broilers fed with a mixture of garlic, onion, peppermint, and thyme showed higher CAT values compared to control groups. Moreover, MDA values were lower in groups fed with rosemary, chamomile or a mixture of rosemary and chamomile. Lipid oxidation is the main non-microbial cause of quality deterioration in meat and meat products (Min, 2005). Lipid oxidation negatively influences the sensory characteristics and the functional characteristics of meat, such as aroma, taste, texture, and nutritional value of meat products. In addition, certain compounds formed during the oxidation of lipids have an adverse effect on the consumer's health due to their mutagenicity, carcinogenicity, and cytotoxicity (Lorenzo et al., 2018). The degradation begins with the sacrifice of the animal and continues progressively until the final product is consumed (Chaijan and Panpinat, 2017). Therefore, all intermediate processes of meat production must be carefully controlled to prevent lipid oxidation reactions (Richards, 2006) minimize the economic losses of the meat industry, and preserving better meat quality and resultant positive impact on consumers' health. Lipid peroxidation or reaction of oxygen with unsaturated lipids produces a wide variety of oxidation products. The main primary products of lipid peroxidation are lipid hydroperoxides (LOOH). Among the many different aldehydes which can be formed as secondary products during lipid peroxidation, malondialdehyde (MDA), propanal, hexanal, and 4-hydroxynonenal (4-HNE) have been extensively studied by Esterbauer and his colleagues in the 80s . MDA appears to be the most mutagenic product of lipid peroxidation, whereas 4-HNE is the most toxic (Esterbauer et al., 1982). Therefore, the studied herbs reduce lipid peroxidation through LOOH reduction with a special impact of chamomile, rosemary, and a mixture of both on lowering MDA production. Further, catalase is one of the most important antioxidant enzymes. Higher catalase (CAT) in meat can reflects better growth performance and the antioxidant capacity, promoting the integrity of intestinal morphology, optimizing the composition of intestinal microorganisms, and upregulating the mRNA expression of tight junction protein (Von Ossowki et al., 1993). Therefore, adding onion, garlic, peppermint, or thyme to the diet would ameliorate several heath aspects of chicks.

Table 6. Effects of dietary herbs on plasma immunoglobulins

Parameters	Plasma immunoglobulins (mg/dl)		
	IgA	IgM	IgG
EG III (Peppermint)	42.85b	2.44b	180.67b
EG IV (Thyme)	38.87b	3.37b	187.30b
EG V (Peppermint + Thyme)	43.64b	3.69b	191.49a
EG VI (Rosemary)	41.25b	3.79b	190.55a
EG VII (Chamomile)	41.55b	4.89a	212.74b
EG VIII (Rosemary + Chamomile)	42.87b	4.62a	192.58b
EG IX (Onion)	41.35b	4.62a	189.21b
EG X (Garlic)	41.68b	4.21a	187.25b
EG XI (Garlic+Onion)	42.98b	4.32a	186.87b
CG Ia (Negative)	53.79a	3.25b	189.32a
CG IIa (Positive)	42.70b	2.27b	187.27a
CG Ib (Negative)	32.71b	2.19b	172.25a
CG IIb (Positive)	37.07b	2.03b	173.68a
SEM	0.67	0.03	1.71

As shown in Table 6, dietary herb supplementation had a significant effect on plasma immunoglobulin levels. Inclusion of chamomile (EGVII), a mixture of rosemary + chamomile (EGVIII) or a mixture of garlic + onion (EGXI) resulted in an increase ($p < 0.05$) in the plasma IgG and IgM levels compared with all other bird groups but did not affect the IgA concentration. Natural products have often been used to stimulate the immune response of the birds. In 2017, Talazadeh and Mayahi reported that adding herbal extracts to broilers diets improved immune responses and have stimulatory effect on the immune system which agrees with our findings. Improved immune response could be attributed to preferential colonization of the gut by the beneficial bacteria and microbial products that improve the function of immunal cells (Janardhana et al., 2009). According to the results of the present experiment, the effects of combined uses of herbal growth stimulants improve the performance of the immune system, which is consistent with the results reported by Ghalamkari et al., (2011) and Houshmand et al., (2012). They state that one of the factors contributing to the improved immune response is the proper growth of lymphoid organs and the subsequent increase in antibody response having a positive correlation with the development of immune system. Their results show that in addition to genetic factors, non-genetic factors such as nutritional supplements in the diet which affect growth can change or modify the expression of the genes responsible for the development of immunity by changing the volume of antibody production and maturation of the immune system.

It was observed that the highest mortality rate (7.8%) during the 1st phase (starter period) of rearing (1-19 days) in the 1st trial was in groups EG III and EG IV whose

birds were fed antibiotics-free and antioxidants-free basal diet as in control group I supplemented with 1**%** peppermint and 1% Thyme dry meals, respectively, followed by groups EG VI (fed antibiotics-free and antioxidants-free basal diet supplemented with 1% Rosemary dry meal), CG IIa (fed basal diet supplemented with antibiotics and antioxidants- Positive control) and CG Ia (fed antibiotics-free and antioxidants-free basal diet-Negative control) attaining the level of 6% each. Whereas the lowest level (2%) was the same in both groups EG VII (Birds were fed basal diet as in control group CG Ia supplemented with 1% Chamomile flower dry meal) and EG VIII (receiving basal diet as in control group I supplemented with 1% dry mixture meal of 0.5% Rosemary and 0.5% Chamomile flowers) followed by birds of group V (receiving basal diet as in control group I supplemented with 1% dry mixture meal of 0.5% Peppermint and 0.5% Thyme) whose livability was at the level of 96.1%.
The influence of Peppermint, thyme and their mixture started to be more effective during the 2nd phase (growing period) resulting in 100% livability in groups EG III- Peppermint and EG IV- Thyme and decreasing to 98% in EG V- Peppermint + Thyme. In addition, no changes were observed in groups EG VI- Rosemary, EG VII- Chamomile and EG VIII- Rosemary + Chamomile. The overall livability (%) at 31 days of age in relation to the initial number of birds was the highest (96%) in EG VII and EG VIII and lowest (88%) in EG VI and CG IIa (90%) as shown in Figure 8. High mortality (between 2 and 7.8 %) in the 1st three weeks of rearing the broiler chicks, was not related to feeding, since all the birds were fed the same antibiotic-free and antioxidant-free basal diet. Moreover, a wide variation in mortality was noticed among groups. Most probably it is related to management and breeding factors since the birds were kept in a local small farm during the feeding trials. In addition, there was no parent history about this local batch that was purchased from a local hatchery. Our results agree with Yassin *et al.* (2009) who concluded that, there is interrelation between 1st week mortality (FWM) at the broiler farms and management factors at the breeder farms (like the breeder age, strain, and feed company of the breeder farms) and at the hatcheries (like egg storage management, hatching management, and season). Additionally, the potential of a chick to survive the first week is directly related to the quality of the day-old broiler (Goodhope, 1991). The day-old chick quality depends on the genetic line of the breeders, breeder age, egg weight, egg storage conditions and duration, and incubation conditions such as temperature, humidity, gas levels, and altitude (Wilson, 1991; Peebles *et al.*, 1999; Vieira and Moran, 1999; Decuypere *et al.,* 2001; Tona *et al.*, 2004, 2005; Decuypere and Bruggeman, 2007). Data achieved during the 2nd phase showed better results in group Ia (no antibiotics and no antioxidants in feeds) where the influence of advancement in age of broiler chicks bringing them natural immunity, thus increasing livability to 97.1%. This reduction in broilers' mortalities in all groups could be explained by improvement in birds' general performance and better build up of the immunity caused by the herb supplements.The obtained results of the trial agreed with the findings of Windisch *et al.*,

(2008) who observed that feed supplements with growth promoting activity increase stability of feed and beneficially influence the gastrointestinal ecosystem mostly through growth inhibition of pathogenic microorganism's growth. Due to improved health status of digestive system, animals are less exposed to the toxins of microbiological origin. Consequently, herbs and spices help to increase the resistance of the animals exposed to different stress situations and increase the absorption of essential nutrients, thus improving the growth of the animals. Moreover, Halliwell *et al.* (1995), Craig (2001), Cetković *et al.* (2004), Skerget *et al.* (2005), Bakirel *et al.* (2008) and Fasseas *et al.* (2008) concluded in their studies that the active components (flavonoids, hydrolysable tannins, proanthocianidins, phenolic acids, phenolic terpenes and some vitamins of E, C and A) of herbs and spices as in rosemary, thyme, oregano, sage, green tea, chamomile, ginko, dandelion and marigold can prevent lipid peroxidation through quenching free radicals or through activation of antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase. In addition, Jacobsen *et al.* (2008) found that rosemary (*Rosmarinus officinalis*) can protect the feed against oxidative deterioration during storage.
Results show that chicks fed 1% rosemary with the basal diet (VI) has numericaly ($p>0.05$) higher values in LBW at slaughter at the end of growth period (31 days of age) as compared with that of the treatment VII and VIII attaining the levels of 1240 g, 1121.1 and 1116.7 g, respectively. In addition the results were higher tha those obtained in control groups (Ia and IIa). This may be due to active compounds that are presented in rosemary green meal inhibiting the excessive growth of harmful intestinal microorganisms, resultimg in positive affect on poultry health and productivity. This is in support with results obtained by Al-Kassie (2008), Kolacz *et al.* (1997), Osman *et al.* (2010) and Sarker *et al.* (2010) that showed a significant improvement of live body weight (LBW) due to the main constituents of the herbs and their essential oils which are responsible for the bulk of the antimicrobial activity (Abaza, 2003; Cross *et al.*, 2007). The Chamomile flowers and Rosemary dry meals play role to enhance the activity of thyroxin hormone that accelerates the nutrients metabolites and biochemical reaction (Mahmmod, 2013). LBW was numerically higher in groups EG VI- Rosemary (1.240 kg/bird) and EG III- Peppermint (1.219 kg/bird) whose birds were fed Rosemary and Peppermint in comparison with other treatments. It is worthy to mention that the lowest numerical levels of LBW were noticed in groups EG VIII- Rosemary + Chamomile (1.117 kg/bird) and EG VII- Chamomile (1.121 kg/bird) followed by CG Ia- Negative control of the 1st trial (1.133 kg/bird) and CG IIa- Positive control of the 1st trial (1.200 kg/bird). This may be due to active compounds that are presented in Rosemary green meal inhibiting the excessive growth of harmful intestinal microorganisms, resulting in good health and productivity nincluding LBW. This is in support with results obtained by Kolacz *et al.* (1997), Al-Kassie (2008), Osman *et al.* (2010) and Sarker *et al.* (2010) that showed a significant improvement of body weight (LBW) due to the main constituents of the herbs and

their essential oils which are responsible for the bulk of the antimicrobial activity (Abaza *et al.*, 2003 and Cross *et al.,* 2007). The Rosemary dry meals play role to enhance the activity of thyroxin hormone that accelerates the nutrients metabolites and biochemical reaction (Mahmmod, 2013) enhancing body growth. Our results agree with the obtained data achieved by Spernakova *et al.* (2007) who have reported that the addition of rosemary powder at 500 mg/kg in poultry diets gave higher body weight gain compared to an unsupplemented control group.

The objective of poultry industry is to offer a tender, juicy, with good flavor and color product and the warranty of a stable shelf-life (Olivo, 1999). The variation in pH of chicken carcasses during *rigor mortis* is an established phenomenon. The pH value in meat was measured using pH meters by direct contact between the sensitive diaphragm of the electrode and the meat tissue. As it can be seen, there is a sharp decrease of pH because of glycolysis, lactic acid formation and the reduction of oxygen liability in muscle. This result agrees with other studies (Cassens, 1994; Schreurs, 2000). Olivo (1999) described that the low rate in pH decline indicates that the animals are not under stress when slaughtered; this is often associated with increased meat tenderness (Ali *et al.*, 1999). Progressive decline in pH was witnessed in all groups but slowest decline in pH was observed in the natural herbal extracts group compared to antibiotic –antioxidant and untreated groups suggestive of less stress in the birds fed natural herbal extracts associated with more tender meat in those group of birds. Our results are in agreement with the findings of Sang-Oh *et al.* (2013) who observed that the pH of the chicken meat did not differ significantly among groups fed different concentration levels of herbs.

Results obtained as average feed intake (FI) at 19 days of age (End of starter period) of the broiler chickens was 657.14g/bird, where no feed rejection was noticed and remaining was negligible. The appetite of the birds was good and the same for all birds of all groups. These results are in contradiction with those achieved of Bassett (2000), Langhout (2000), Kamel (2001), Williams and Losa (2001) and Hermandez *et al.* (2004) who declared that feed intake was increased with addition of essential oils derived from spices and herbs relating this to the fact that, this improvement in feed consumption was observed may be due to the appetizing effect of active ingredient (borneol) in rosemary (Cabuk *et al.*, 2003) but in agreement with Panda (2005) who found that Chamomile improves FI as it contains active ingredients (azulene, flavonoid and coumarin glycosides and fatty acids) giving anti-inflammatory, antiseptic, carminative, diaphoretic, sedative properties. Our results agree with the obtained data achieved by Spernakova *et al.* (2007) who have reported that the addition of Rosemary powder at 500 mg/kg in poultry diets gave higher body weight gain compared to an unsupplemented control group leading to better FCR. The improved daily live weight gains and feed conversion ratio of birds fed the diets containing Pepperiment 1% and Rosemary 1% in this study agreed with the results reported by Al-Kassie (2008). It laso agree with the results of Osman *et al.* (2005) who reported that addition of 200 ppm essential oil mix derived from oregano, Clove and anise improved body weight and feed conversion ratio compared to control groups in broiler.Better numerical results in LBWG was obtained at 20-31 days of age in groups EG VI- Rosemary (729.71±63.70g) followed by CG IIa- positive control of the 1st trial (719.5 ± 399.83g), EG III- Peppermint (691.25±235.43g), EG IX- Onion (682.0±125.70g) and EG V- Peppermint + Thyme (676.20±145.54g). Our results disagree with the findings observed by Santurio *el al*. (2007) who declared that the improvement of body weight gain and feed conversion are related to Chamomile powder inclusion due to the effect of some active compounds found in Chamomile flowers which had different effects against microorganism antimicrobial, antifungal and antioxidant and anti-inflammatory effects. In general our results agreed with the obtained findings of Windisch *et al.* (2009) who noted that phytobiotic feed additives as oil extract reduced feed intake with no changes in body weight and daily weight gain and improved feed conversion ratio in broilers. The application of dried herbs and spices increased feed intake, decreased body weight, increased average daily weight gain and improved feed conversion ratio. Feed consumption was significantly ($P<0.05$) increased (VI, VII) for grower phase. Increased feed consumption may be attributed to flavoring effects of 1% Rosemary (VI) and 1% Chamomile (VII) which improve the palatability of feed due to the chemical components and flavor of the chamomile flower as compared to I and II (control groups).These results are in accordance with those of Bassett (2000); Langhout (2000); Kamel (2001); Williams and Losa (2001) and Hermandez *et al.* (2004) who found that feed intake was increased with addition of essential oils derived from spices and herbs. In addition, this improvement in feed consumption was observed may be due to the appetizing effect of active ingredient (borneol) in rosemary (Cabuk *et al.*, 2003) and Chamomile (azulene, flavonoid and coumarin glycosides and fatty acids) giving anti-inflammatory, antiseptic, carminative, diaphoretic, sedative properties (Panda, 2005). Chamomile flowers inhibit the harmful intestinal Microorganisms, thus counter acting excessive growth (Kolacz *et al.*, 1997). Feed conversion at the end of starter phase 1-19 days attained inferior levels with the addition of Rosemary dry meal in comparison with control diets 1.60, 1.47 and 1.43 respectively. In grower phase that inclusion of rosemary to the basic diets showed a superior shift in this indicator attaining the level of 1.08 followed by thyme (1.26) and pepperiment (1.27) inclusions while the inferior level (1.87) among all groups was observed in EG VII- Chamomile surpassing Control Groups Ia (1.57) and IIa (1.51). The improvement and drawback in body weight gain and feed conversion are due to the effect of some active compounds included in some of te herb-ingredients added to the basal diet having different effects against microorganism antimicrobial, antifungal and antioxidant and anti-inflammatory effects (Santurio *el al.*, 2007). In this study, the addition of thyme showed significant difference only in the FCR from 20 days to 30 days old. This result confirmed the ones from Abdulkarimi *et.al.* (2011), as they noted that FCR did improved by adding thyme in drinking water and matched the results of El-Ghosein and El-

Beitawi (2009) that formulated diets by adding 0.5, 1.0, 1.5 and 2.0% of crushed thyme. Rahimi *et.al*. (2011) showed similar results as the addition of plant extracts showed significant difference in broiler performance. This could be due to the volatile oil components of thyme (thymol, carvacolo, borneol and geraniol). Thymol is the most important and primary volatile oil of thyme. Those oils and components of thyme affect the performance of the digestive system by enhancing the secretion of enzymes and endogen (amylase and chemotripsin). By this, the absorption rate of the intestine will increase and will positively affect the FCR and general performance of the birds (Feizi *et.al*., 2013). However, through the whole experiments, FCR did not show any significant difference between the different groups. Those results where matching with the experimental outcoming of Hernandez *et.al*. (2004) that concluded that the addition of thyme and herbals did not affect significantly the performance of broiler chickens. In contradiction, Al-kassie (2009) that reported significant difference between different groups and the addition of thyme. The average live body weight, live body weight gain, weight of edible organs and breast weight did not show any significant difference through the whole experiment. Those outcomes differ from the results of Al-Kassi *et.al.* (2010). On another hand, Shabaan (2012) reported that the addition of thyme led to an insignificant different in body weight gain and feed intake at the grower period (9-28 days) however during the finisher period (29-42 days) there were significant difference between values of body weight gain, feed intake and feed conversion. Amouzmehr *et.al*. (2012) declared that the addition of thyme did not affect the performance of the broilers regarding the feed intake, weight gain and feed conversion ratio. These results matched the outcome of this experiment. This insignificant difference in most of the performance traits measured may be the consequence of the farming system adopted to raise the flock as open farm system, and manual feeding and drinking may lead to less efficacy performance records and punctual survey of the flock regarding feed intake and feed conversion eventually.

For the edible organs and carcass yield, the result of this experiment matched those of Sarica *et.al*. (2005)'s experiment where the effects of the supplementation of thyme powder did not have significant difference on the weights of internal organs as heart and liver.

As illustrated by Garcia *et al.,* (2010) that color is one of the main indicators of the quality of most foods. This sensorial quality has a high influence of the meat purchase decision and its acceptance by consumers. It is an important functional quality and it is closely related to other qualities, such as pH, water holding capacity, emulsifying capacity, and texture. In most cases, color can be considered as an indicator of these properties, which together, will affect consumer behavior, and will determine handling characteristics, tenderness, juiciness, aspect, yield, and cost of meat products. Barbut (1997) noted that L* value is the main parameter that determines poultry meat color. The optimal lightness range (L*) of chicken and turkey fillets is around 49-50. Higher values indicate lighter color, indicating that fillets have low pH (pH<5.6), whereas values below that range indicate that fillets are darker and have high pH (pH>5.9). The main scope of animal production is to ensure the high productivity, healthy animals and quality animal products, which are stable and appropriate for further processing. In this aspect, herbs and spices are not just appetite and digestion stimulants, but can, with impact on other physiological functions, help to sustain good health and welfare of the animals and improve their performance. Current studies show promising results regarding the use of phytochemicals as growth and production promoters. There is still a need to clarify the phytochemical composition and the mechanisms of action for many herbs, spices and their extracts and furthermore, to assess the appropriate dose that should be safely used in specific circumstances and animal species. Carroll *et al.* (2012) stated that environmental temperature of poultry houses, in general, is not effectively controlled. Therefore, poultry production suffers huge losses due to heat stress, which is caused by high temperatures in many areas during the summer. Therefore, poultry often suffer from heat stress, which causes inestimable and negative impacts on their growth, development, production, and reproduction (Liu and Peng, 2001). The behavior of chickens can significantly influence their growth rate, consequently influencing production costs. Young chicks present high metabolism rates. While their growth rate is fast, their ability to adapt to the changes in environmental conditions is poor. Moreover, since they do not present sweat glands in the skin, chickens are highly sensitive and vulnerable to heat stress, particularly when they are young. (Neves *et al.*, 2010; 2014). This may explain the mortality cases in this experiment since it was done in July 2017, in a semi-arid region, where a heat stress stroke leading the temperature to reach high degrees (46 ˚C). Sharifi *et al.,* (2013) stated that medicinal plant supplements are used commonly as dietary additives for humans. They are chosen for their non-toxic chemical composition, relatively low cost and easy availability. Also, over the past few years, medicinal plants and their extracts have been used in animal diets as feed additives in order to improve their performance, health and the quality of their products. Hernandez *et al.* (2004) stated that the supplementation of poultry diets with aromatic plants have a stimulating effect on digestive system of the animals through increase in the production of digestive enzymes and by improving the utilization of digestive products through enhanced liver function. Present results are in agreement with the finding of Cabuk *et al.* (2006) in the importance effect of active substances in the medicinal and aromatic plants as an active substances and digestive stimulators, also its effect as antimicrobials, especially the intestinal microbes that located in the digestive system. The inclusion of garlic powder in diet of broiler significantly ($P<0.05$) enhanced the body weight and the weight gain as compared to the control group. The improved weight gain of bird fed on garlic powder could be attributed to allicin active ingredients in garlic which promotes the performance of intestinal flora, thereby improving digestion and enhance the utilization of energy, which improve the growth of birds. This result was in line with the finding of ElGamry *et al.* (2002); Tollba and Hassan (2003); Al-Homidan

(2005); Fayed *et al.* (2011) and Safa *et al.* (2014). The results also coincided with the finding of Ahmad (2005) who found higher weight gain of broiler chicks fed on ration supplemented with garlic as natural growth promoter. Moreover, the present experiment showed that addition of mint to broiler feed increased live body weight and live body weight gain. This comes in line with Sharifi *et al.* (2013) who stated that a significant growth promoting effect was observed from flavomycin and peppermint feed additives during the grower and finisher periods. The higher body weight gain observed in broilers fed with peppermint diet may be related to the reported properties of menthol (Lovkova *et al.*, 2001). Groups taking 1% garlic powder and 1% peppermint showed better live body weight and live body weight gain. These results came in line with Miloševic *et al.* (2013) who reported that supplementation of 1.5% and 3.0% of garlic powder had a positive effect on body mass and difference between treatment and control group was statistically significant. Also Ramiah *et al.* (2014) recorded that broiler chickens fed basal diet with 0.5% garlic powder had significantly higher ($P<0.05$) weight gain compared to the control. This agrees with Puvača *et al.* (2014), who showed that broiler chickens with 0.5% garlic powder in diet had higher body weight with statistically significant differences ($p<0.05$) compared to the control treatment. Moreover, Oleforuh-Okoleh *et al.* (2014) stated that inclusion of garlic and ginger had strong effect on the growth performance traits monitored. Though there was no significant difference in the initial body weight of the birds, feeding the test ingredients significantly affected the final body weight of the birds. The significant increase in body weight gain of birds fed garlic confirms the findings of Demir *et al.* (2003), Ademola *et al.* (2005) and Javendel *et al.* (2008) who fed herbal plants (ginger and garlic) as growth promoters in broiler diets and observed a pronounced improvement in their body weight gain and feed conversion ratio. These results might be due to the good health status of the birds, which may be caused by the addition of garlic, and might also be due to the chemical composition of garlic Onibi *et al.* (2009) and Fadlalla *et al.* (2010). Ramakrishna *et al.* (2003) also suggested that garlic supplementation enhances the activity of pancreatic enzymes and provides an environment for better absorption of nutrients. Feeding the test ingredients in powder and through infusion, recorded a significant difference in the final body weight, and feed conversion ratio which is contrary to what is cited in Al-Moramadhi (2010), who reported a non-significant effect on body weight of birds fed ginger in powder form. Birds fed the test ingredients in powder form had a significant increase on their daily body weight gain and daily feed intake than those fed the dietary treatment through infusion. In addition, in this study, the addition of 1% mint and 1% garlic showed significant difference only in the FCR from 1 day to 25 days old. This result confirmed the research of Tollba and Hassan (2003) reporting that garlic improved broiler growth and feed conversion ratio (FCR) and decreased mortality rate. Onu (2010) showed that ginger and garlic supplementation at 0.25% level in broiler finisher diets enhanced the conversion ratio of the birds and the best performance was attained by the group of birds fed on diet containing 1% garlic powder that had the best feed conversion efficiency (Elagib *et al.*, 2013). In contrast with the research of Raeesi *et al.* (2010) that showed that the feed intake significantly tended to be higher in the birds fed on garlic powder diets compared to the control group, current results showed that the birds fed 1% of garlic powder showed the least feed intake whereas the group taking antibiotics showed the highest feed intake. The relative weights of liver and heart were not affected by the supplements. These results are consistent with those of Hernandez *et al.* (2004), Sarica *et al.* (2005) and Cabuk *et al.* (2006) who concluded that antibiotic growth promoters or herbal powders/essential oils did not change the relative weights of some internal organs. Moreover, there was no significant difference between the treatments in the tenderness which was proven by Safa *et al.* (2014) who stated that no significant differences were observed between all treatment groups in subjective meat quality attributes (color, flavor, juiciness and tenderness) of the breast and thigh meat. All scores are at above moderate values. These results didn't agree with Singh *et al.* (2015) who reported that supplementation of whole bulb garlic powder at 1.0, 1.5 and 2.0 % supplementation levels lead to better appearance, color flavor, tenderness, juiciness and overall acceptability then control and Antibiotic fed groups. The positive influence of garlic supplementation on meat can be correlated to the presence of volatile compounds, phenolic content and antioxidant active compounds (Canogullari *et al.*, 2010), in addition to S containing enzymes, amino acids and minerals (Newall *et al.*, 1996). Research findings indicated that pH plays a significant role in the extent of microbial spoilage (Ngoka and Froning, 1982). Glycogen concentration in muscle is the main factor on which pH relies (Sallam *et al.*, 2004). It was worth to mention that no significant difference was shown between the different supplements. This was in contrast to the results of Holden *et al.*, 1998 who stated that pH values of chicken sausage can be increased by the treatment of garlic. Meat having higher pH, holds more water during storage and will produce more juice after meat preparation. If more juice is produced from the meat then it will give juicier, more succulent and tender eating experience. Nevertheless, the variations between the results of the present study and those obtained by some other researchers could be attributed to differences in the environment, source and composition of garlic and peppermint used, preparation process, feed inclusion levels, the overall diet composition and breed/strain of bird used in the study.

There is no doubt that antibiotics play an important and essential role in the production and animal health but at the same time may negatively affect the health of consumers. The use of antibiotics as growth promoters has led to the development of bacterial resistance, on the other side it may also result in residual problems in the tissues of birds and animals. The dangers posed by the development of resistance in poultry livestock and human beings have been documented (Mmereole, 2010; Sarker *et al.*, 2010). As a result of banning growth-promoting antibiotics in European Union and many other countries by 2006 livestock producers need alternatives to be found to improve the healthiness and safety of poultry products in order to minimize the loss of productivity and performance and negative impact on economic terms and for that several kinds of antibiotics alternative developed and used currently, it has been found that natural additives such as herb and medical plants have some properties as growth enhance to replace synthetic drugs, the antimicrobial effect of the medical plants is well documented (Cowan, 1999; Abdulla *et al.*, 2011). Mountzouris *et al.* (2009) showed that phytogenic effects have been proven in poultry for feed palatability and quality (sensory aspects), growth promotion (improved weight gain and feed conversion ratio, reduced mortality), gut function and nutrient digestibility (improved growth), gut microflora (less diseases of the gut, improved growth, reduced mortality), immune function (improved health), and carcass meat safety and quality (reduced microbial load, improved sensory). Similar results were reported by Ayad *et al.* (2007) who found no significant differences among all treatments in dressing percentage of broiler chickens after supplementing the diets with herbs. These findings agree with the results obtained by Grashorn (2010) who concluded that breast meat yield was higher in groups where herbs and spices were added to diets without antioxidants and antibiotics. Even though our result was insignificant in more than one treatment, but it agrees with the findings of Mahmmod (2013) that showed significant increase in weight of breast in treatments of broiler chickens receiving herbs in comparison with control group suggesting that such promoters might be used in broiler diets, since they do interfere positively on the yield of the most commercialized edible cuts (breast, legs and wings). All results obtained were insignificantly different and almost the same where it calibrated from 21.9 ± 0.96 % (treatment EG VII) and 21.8 ± 0.9 % (treatment (EG VIII) to 21.2 ± 0.64, 21.3 ± 0.73 and 21.4 ± 1.44 % in treatments CG Ia, CG IIa and EG VI, respectively. There were no significant differences among of all treatments in dressing percentage and edible visceral ratio in broiler chickens, similarly results were reported by Ayad *et al.* (2007). Results showed that there were insignificant differences among carcass net weight (%), The addition of 1% Rosemary dry meal, 1% Chamomile flower meal and 1% mix of both meals to the basal diet showed no significant increase in weight of of breast, liver and heart compared with other treatment group. It is suggested that such promoters might be used in broiler diets, since they do not interfere negatively with the yield of the most commercialized edible cuts (Net carcass weight, breast and liver and heart).

V. WORK CONTRIBUTIONS

This work presents both scientific and practical contributions:

1. The study presented cost-effective methods to ameliorate broiler chicken performance using natural herbs widely available at almost zero cost in their feed, therefore reducing the cost of meat production in Lebanon.
2. It offers a strategy to ameliorate the production conditions of the poultry sector in Lebanon, coping with the high cost of traditionally imported antibiotics and their negative impact on human health in addition to their scarcity in the Lebanese market due to the economic crisis nowadays.

3. Moreover, the study showcased how natural herbs in poultry feed can provide a growth promoting effect, essential for improving production parameters and diseases prevention.
4. The study determined positive effects of natural herbs inclusion in the broiler chicken diet, such as enhanced palatability for feed (chamomile), feed conversion (rosemary, thyme), and body growth (rosemary), as well as ameliorated immunity (peppermint, thyme, chamomile, garlic, onion) and blood profile (rosemary)
5. Adding to the separate positive effects of natural herbs, the study contributed some new positive findings firstly reported concerning the combined effects of some herbs, whether on chicken body growth (thyme+ peppermint) or immunity system (garlic + onion, chamomile + rosemary).

CONCLUSION AND RECOMMENDATIONS

The objective of the present study was to compare the effects of the supplementation of six herbal natural feed additives with and without antibiotics and antioxidants to soybean-corn-based broiler diets on growth performance and carcass parameters.

It is highly recommended to use 1% Rosemary and 1% Chamomile flowers meals as a supplementation to 100 kg of basal diets in the daily feeds of broiler chickens in the growing period. In addition to the achievement of results observed in this trial it is highly requested to test these herbs in diets fed in the finisher period to explore the actual effect of herbs on slaughter performance of chicks.

The addition of thyme, peppermint, garlic and onion powders have a potential to enhance the performance of broiler chickens. Even though most of the traits did not show significant difference in this experiment, but still positive numerical results were recorded compared to the control group, which can highlight that those herbs can be included in broiler feeding rations and replace antibiotics as growth promoters.

The inclusion of a mixture of chamomile and rosemary increased PVC, hemoglobin, WBC, eosinophils, total protein, albumin, ALT, HDL, LDL and lowered LOOH and MDA. Moreover, supplementing the diets with rosemary increased heterophils, lymphocytes, monocytes and mean corpuscular hemoglobin while chamomile usage increased globulin concentration.

IgG and IgM increased following the supplementation of the diets with both mixtures' chamomile/rosemary, garlic/onion.

For better experimental study, and to obtain more specific and accurate results on the use of aromatic plants as natural antioxidants and growth promoters, further studies are needed with higher inclusion rate of thyme, peppermint, rosemary, chamomile, garlic powder and onion powders per ton of finished feed, and to raise flocks in more environmental controlled farm to eliminate loses of net energy due to energy loss for maintenance and control of body temperature, and use of automatic feeders and drinkers to limit the waste of feed by the birds and narrow the error of feed intake and feed lost records.

Conference papers

1. **Al Hanna R**, Genova K, Al Jammal B (2018) Impact of feeding herbs – peppermint and thyme on broiler chickens body performance during the growth period. AGROSYM proceedings

2. **Al Hanna R**, Genova K, Jammal B (2018) The impact of feeding with herbs rosemary and chamomile on broiler chickens body performance during the growth period AGROSYM proceedings

Journal papers

3. **Al Hanna R** (2022) The Impacts of Locally Cultivated Herbs on Physical Parameters and Meat Quality of Broiler Chickens. J. World Poult. Res., 12 (3): 171- 180. DOI: https://dx.doi.org/10.36380/jwpr.2022.20