بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Response of Broiler Chicks Fed on Graded Dietary Essential Oils Combination as Natural Feed Additives

إستجابة كتاكيت اللاحم إلى خليط بمستويات متدرجة من الزيوت الأساسية كإضافة علفية طبيعية

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بسم الله الرحمن الرحيم

قال الله تعالي:

يَا أَيُّهَا الَّذِينَ آمَنُوا إِذَا قِيلَ لَكُمْ تَفَسَّحُوا فِي الْمَجَالِسِ فَافْسَحُوا يَفْسَحِ اللَّهُ لَكُمْ ۖ وَإِذَا قِيلَ انْشُزُوا فَانْشُزُوا يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ ۚ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴿11﴾

صدق الله العظيم

سورة المجادلة الآية 11

Dedication

This work is dedicated to everyone have a site in my heart

Ongoing charity for my beloved brother

Elrasheed Mustafa Mohammed Elsinnary

(May God rest his soul)

Fatima

Illustration

The study put into final form in this thesis has not been submitted for others degree or diploma for any other examining body. Except where acknowledgment is made by references, the work mentioned herein was the un-aided efforts of the author.

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My great thanks to my God for giving me a good patience and high power to complete my studies and come to light.

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ABSTRACT

Four experiments were fulfilled out to appraise the response of broiler chicks to diets supplemented with graded levels of mixed essential oils extracted from herbal plants including: Eucalyptus leaves (Eucalyptus Essential Oil, E.E.O) (Myrtaceae family), Clove fruits (Clove Essential Oil, C.E.O) (Eugenia ssp), Basil plants (Basil Essential Oil, B.E.O) (Ocimum basilicum L.) and a combination of them (1:1:1) as natural feed additives.

96 day old, un-sexed broiler chicks (Cobb strain), were used in each experiment. Chicks were divided into four groups of (24) chicks in each experiment and each group was sub-divided into three replicates with (8) birds in each. The first group was fed on control diet supplemented with Eucalyptus and Clove mixed essential oils at levels of (0.00, 200 mg/kg; 400 mg/kg, and 600 mg/kg), the second group was fed on control diet supplemented with Eucalyptus and Basil mixed essential oils at levels of (0.00, 200 mg/kg, 400 mg/kg, and 600 mg/kg), the third group was fed on control diet supplemented with Clove and Basil mixed essential oils at levels of (0.00, 200 mg/kg, 400 mg/kg, and 600 mg/kg), the fourth group was fed on control diet supplemented with Eucalyptus, Clove and Basil mixed (1:1:1) essential oils at the same levels mentioned above. The experiment parameters covered growth performance, carcass and non-carcass values, sensory evaluation, serum constituents, enzyme activities and minerals and economical appraisal.

The results were showed that no significant effects in body weight, body weight gain, feed intake and feed conversion ratio among all tested groups, however inclusion of mixed essential oils at (200 mg/kg) had the best results compared with control group, also the results declared that there were no significant differences among all tested groups in internal organs, commercial

cuts; their separable tissues, the subjective and objective of meat quality parameters. Also the same results were observed for serum metabolites, enzymes activities and minerals ratios. The results of economical evaluation of the tested groups supplemented with mixed essential oils in broilers diets declared economical values. (200 mg/kg) of Eucalyptus and Clove mixed essential oils registered profitability ratio (1.149), while (200, 400 and 600 mg/kg) Eucalyptus and Basil mixed essential oils registered (1.140, 1.147 and 1.112) profit ratio as successively, also (200, 400 and 600 mg/kg) Clove and Basil mixed essential oils registered (1.222, 1.008, 1.134) profit ratio as successively, and finally (200 mg/kg) of Eucalyptus, Clove and Basil mixed essential oils registered profitability ratio (1.202).

The state of being organized, (200 mg/kg) of Clove and Basil mixed essential oils registered (1.222), followed by (200 mg/kg) of Eucalyptus, Clove and Basil mixed essential oils which registered profitability ratio (1.202), then (200 mg/kg) of Eucalyptus and Clove mixed essential oils registered profitability ratio (1.149) and (400 mg/kg) of Eucalyptus and Basil mixed essential oils registered (1.147) whereas (200 mg/kg) of Eucalyptus and Basil mixed essential oils recorded (1.140).

As general we can record that, supplementation of mixed essential oils to broiler diets as growth promoters had good beneficial economic effects.

المستخلص

تم اجراء أربعة تجارب لتقييم مدى اســـتجابة كتاكيت الدجاج اللاحم لعلائق تحتوى على مستويات متدرجة لخليط من الزيوت العطرية المستخلصة من النباتات الطبية (اوراق الكافور، ثمار القرنفل ونبات الريحان) كخليط من تلك الزيوت بنســبة (1:1:1) تم اســتخدام عدد 96 كتكوت لاحم غير مجنّس من سلالة الكوب فى كل تجربة، ووزعت عشوائيا الي مجموعة قياسية وأربع مجموعات تجريبية، بكل مجموعة 24 كتكوت، وقسّمت كل مجموعة الى ثلاث مكررات بكل منها ثمانية كتاكيت (4×3×8). قســمت مجموعة التجربة الأولى الى أربع معاملات، غذيت المعاملة الأولي علي العليقة القياســية مضـــافا اليها خليط من زيت الكافور مع زيت القرنفل بنســبة (0.00, 200, 400 و600 ملجم/كجم) علي التوالي. وقســـمت مجموعة التجربة الثانية الي أربع معاملات أيضـــا غذيت علي العليقة القياســية مضـــافا اليها خليط من زيت الكافور مع زيت الريحان بنســبة (0.00, 200, 400 و600 ملجم/كجم) علي التوالي. كما قســـمت مجموعة التجربة الثالثة الي أربع معاملات غذيت علي العليقة القياسـية مضــافا اليها خليط من زيت القرنفل مع زيت الريحان بنسـبة (0.00, 200, 400 و 600 ملجم/كجم) علي التوالي. وقســـمت مجموعة التجربة الرابعة الي أربع معاملات غذيت علي العليقة القياسية مضـافا اليها خليط من زيت الكافور مع زيت القرنفل مع زيت الريحان بنسبة (0.00, 200, 400 و600 ملجم/كجم) علي التوالي.شـــملت القياســـات للتجارب الأداء الانتاجي، قيم الذبح والذبيح، نسب الأعضاء الداخلية، نسبة الأملاح ونشاط الانزيمات، تحليل الدم والقطع التجارية ونسب اللحم وصفاته الانطباعية واختبارات التذوّق ثم الدراسة الأقتصادية. أظهرت النتائج المتحصّل عليها بأن اضــافة الخليط من الزيوت النباتية بنسـب متدرجة (200, 400, 600 ملجم/كجم) عليقة ليس له أثر معنوي في قيم الوزن الحي والوزن المكتسـب والعليقة المسـتهلكة ومعدل التحويل الغذائي، الا أن اضافة الخليط بنسبة (200 ملجم/كجم) عليقة كان الأفضل علي الأطلاق.

كما أشــارت النتائج أيضـــا الي عدم وجود فروقات معنوية لجميع المعاملات وبنســبها المختلفة علي الأجزاء الداخلية وأنسجة اللحم وصفاته الكيميائية والنوعية والأنطباعية والقطع التجارية. أيضا تحليل اللحم الكيميـائي لم يظهر تأثير معنوي في مســـتوي الكلســـترول والبروتين وحامض اليوريك ، كما ينســحب القول علي مســتوي الأملاح ونشــاط الأنزيمات في المعاملات المختلفة مقارنة بالمجموعة القياســـية.أما التقييم الأقتصـــادي فقد أظهر نتائج واضـــحة ، وأثبت أن اضـــافة الخليط من الزيوت وبمسـتويات متدرجة له فوائد اقتصــادية، حيث سـجلت (200 ملجم/كجم) من خليط زيت الكافور مع

زيت القرنفل نســبة ربحية (1.149)، بينما ســجلت (200, 400 و600 ملجم/كجم) من خليط زيت الكافور مع زيت الريحان نسبة ربحية (1.140, 1.147, 1.112) علي التوالي، كذلك سجلت (200, 400 و600 ملجم/كجم) من خليط زيت القرنفل مع زيت الريحان نســـبة ربحية (1.222, 1.008, 1,134)علي التوالي، كما سجلت (200ملجم/ كجم) من خليط زيت الكافور مع زيت القرنفل مع زيت الريحان نسبة ربحية (1.202).

ترتيبا ســجلت (200 ملجم/كجم) من خليط زيت القرنفل مع زيت الريحان أعلي نســـبة ربحية (1,222), يتبعها (200 ملجم/كجم) من خليط زيت الكافور مع زيت القرنفل مع زيت الريحان حيث ســجلت نســبة ربحية (1.202), ثم يليها (200 ملجم/كجم) من خليط زيت الكافور مع زيت القرنفل حيث ســجلت نســبة ربحية (1,149), و(400 ملجم/كجم) من خليط زيت الكافور مع زيت الريحان حيث سجلت نسبة ربحية (1,147) بينما سجلت (200 ملجم/كجم) من نفس المجموعة نسبة ربحية (1,140).

لذا لنا أن نفيد بأن إضــافة الخليط من الزيوت ســالفة الذكر كإضــافات علفية مفيدة في علائق الدجاج اللاحم.

Chapter One

Introduction

The poultry industry is very important economically in many countries such as our country SUDAN. The rest of the poultry farmers are focusing on broiler production due to its less space requirement, smaller marketing age, higher weight gains and quick returns.

The poultry industry is currently moving toward reducing the use of antibiotics because of increased concern about antibiotic-resistant bacteria and antibiotic residues in meat and eggs Mashayekhi *et al.,* (2018), and for that reasons use of antibiotics as growth promoters was banned in the European Union for poultry production in (2006), so several management and nutritional strategies in poultry industry were proposed in order to maintain high standards of productivity, healthiness, and welfare (Stevanovi´c *et al.*, 2018; Ognik *et al.*, 2016 and Florou-Paneri *et al.*, 2019).

To improve chicken healthiness and to fulfill consumer expectations in relation to food quality, poultry producers nowadays commonly apply natural dietary supplements mainly medical, aromatic, and spice herbs (Popović *et al.*, 2018). And due to essence, flavor, antimicrobial, and preservative properties, plant secondary metabolites have been used by mankind since early history, (Giannenas *et al.,* 2020; Akram *et al.,* 2019 and Jalal *et al.,* 2019).

The species, herbs, vegetables, some of the plant and growth promoters in broiler diets to the improvement of efficiency of the growth conversion and reducing the cost of feed, adding herbal plants as growth promoters in broiler diets will improve in their feed conversion ratio, less mortality rate, body weight gain (Borazjanizadeh *et al.*, 2011). So natural feed additives extracted from herbs, plants, and spices such as essential oils have been evaluated and considered as a substitute to antibiotic and chemical feed additives in livestock productions for improving animal production and health.

Essential oils are volatile oils obtained from plants. Essential oils are very complex compounds mixtures and their chemical composition and concentration of individual compounds are variable. Essential oils are found to have anti-bacterial property and also exhibiting anti-oxidant, anti-inflammatory, anti-carcinogenic, digestion stimulating and hypolipidemic activities. The impact of pH on the biological activity of essential oils and their ability of affecting the bacterial growth of some un-desirable bacterial species makes them ideal candidates as gut bacterial modulators. The anti-microbial mechanism of essential oils is their lipophilic properties and chemical structures. Essential oils not only can they function individually, but their effects can also be enhanced through synergistic effects both between individual essential oils and in a combination with others. Essential oils hold the potential of possible therapeutic exploitation in different ways in animal production. They represent a wide range of biologically active compounds like phenolic and terpenoids which possess a variety of functions with health-related benefits and nutrigenomics implications on the development of the gut and immunity (Christaki *et al.*, 2020). Application of essential oils in animal feed for health management, improvements in productivity and quality has proved a viable strategy, which is also the consumer's demands.

For all mentioned above, the objective of our present studies were to examine the effects of dietary supplementations of mixed essential oils at graded levels on the growth performance, carcass traits and blood serum constituents of broilers.

CHAPTER TWO

LITERATURE REVIEW

2. 1 Feed Additives:

The international feed industry is facing the challenge of the awareness among the consumers of meat on the risk of bringing about antibiotic resistance in pathogenic micro biota through antibiotics used in animal and poultry feeds. It has directed them towards the non-antibiotic feed additives. Among them, the feed additives of plant origin, called as Phytogenic Feed Additives (PFA) or Phytobiotics or Phytoadditives are considered to be a better alternative as non-antibiotic growth promoters, even though there are well established non antibiotic growth promoters such as organic acids and probiotics. The Phytogenic feed additives also vary widely in their botanical origin, processing and composition. They have been used in solid, dried and ground forms or as extracts or essential oils (Guo *et al.*, 2003).

2. 2 Essential Oils:

Essential oils are aromatic, oily extraction from different parts of a plant, like flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits or roots. The term "Essential oil" is being derived from '*Quinta essential'* of the major plant families, coined in the sixteenth century by Paracelsus von Hohenheim for the effective component of a drug (Burt, 2004). Essential oils are formed in plants from isopentenyl pyrophosphate units which are polymerized yielding a large variety of components. Essential oils having complex interactions of different classes of compounds such as, aldehydes, ketones, phenols, esters, alcohols, hydrocarbons or ethers in plants (Krishan and Narang, 2014). The use of essential oils in enhancing productivity may give promising effects as growth and health promoter. Essential oils derived mainly from spices and herbs and their pure compounds have been shown to have antimicrobial activity, antioxidants, hypocholesterolemic, and digesting effects (William, 2001; Jang *et al*, 2004). The supplementation of essential oils to poultry diets have shown to stimulate the production of endogenous enzymes and thus enhances feed utilization (Jang *et al*, 2004). It has been also shown that the dietary incorporation of herbs and their associated essential oils may provide beneficial effects on poultry performance and health due to the antimicrobial activity of their phytochemical components (Lee *et al*., 2004). Most of the essential oils which are phenolic in nature act on bacterial cell by increasing cell permeability which leads to cell death due to water imbalance and hence show antibacterial action. Antibacterial activities of essential oils were also well documented (Tiihonen *et al*, 2010). Essential oils also promotes feed intake, digestive enzyme production and destroy pathogens in poultry (Lee *et al*, 2003).

2. 2. 1 Composition of Essential Oils:

Essential oils or volatile oils are aromatic oily liquids extracted by distillation from plant parts, such as flowers, buds, seeds, leaves, twigs, bark, wood, fruits and roots. The term 'essential oil' can be regarded as a poorly defined term, a by-product of medieval pharmacy, and for this reason, the term 'volatile oil' has been proposed as an alternative. Nevertheless, the former term 'essential oil' is used more often. Essential oils are characteristic for their strong smell and varied composition. Chemically, essential oils are complex and highly variable mixtures of constituents that belong to two groups: terpenoids (monoterpenes and sesquiterpenes), aromatic compounds (aldehyde, alcohol, phenol, methoxyderiva-tive, and so on) and terpenoids (isoprenoids) (Bakkali *et al*., 2008; Nazzaro *et al*., 2013). They are characterized by two or three major components at fairly high concentrations (20–70%) compared to other components present in trace amounts. This determines their versatile biological activity, which is to a large extent conditioned by the dominant component. Various essential oils have many properties in common, e.g. they can be vaporized with steam, are lipophilic, liquid at 18^{o}C, optically active, and well soluble in ethanol, propylene glycol, or in lipids (Gopi *et al*., 2014). Compounds and aromas of essential oils can be divided into two major groups: terpene hydrocarbons and oxygenated compounds. Hydrocarbons are molecules composed of H and C atoms arranged in chains. These hydrocarbons may be acyclic, alicyclic (monocyclic, bicyclic, or tricyclic), or aromatic. Terpenes are the most common class of chemical compounds found in essential oils. Terpenes are made from isoprene units (several 5-carbon base units, C5), which are the combinations of 2 isoprene units, called 'terpene units'. Essential oils consist mainly of monoterpenes (C10) and sesquiterpenes (C15), which are hydrocarbons with the general formula (C5H8) n. Diterpenes (C20), triterpenes (C30), and tetraterpenes (C40) exist in essential oils at low concentrations. The oxygenated compounds are the combination of C, H, and O, and there are a variety of compounds found in essential oils. Oxygenated compounds can be derived from terpenes, in which they are termed 'terpenoids'.

2. 2. 2 Essential Oils Properties:

2. 2. 2. 1 Physical Properties of Essential Oils:

Essential oils could be obtained through various methods like fermentation, extraction or expression; however, steam distillation is used as the most common method for commercial purpose. The Essential oils possess characteristic odor, and are soluble in organic solvents. Most of the oils are lighter than water with a specific gravity between 0.8-1.17. These oils are sensitive to heat and light, therefore should be stored in dark bottles and cool places.

2. 2. 2. 2 Anti-Microbial Activity of Essential Oils:

Essential oils have long been recognized because of their anti-microbial activity (Smithpalmer *et al*., 1998; Hammer *et al*., 1999). The exact anti-microbial mechanism of essential oils is their lipophilic property Conner, (1993) and chemical structure Farag *et al*., (1989) could play a role. Terpenoids and phenylpropanoids can penetrate the membrane of the bacteria and reach the inner part of the membrane because of their lipophilicity (Helander *et al*., 1998), but it has also been proposed that structural properties, such as the presence of the functional groups Farag *et al*., (1989), and aromaticity Bowles and Miller, (1993) are responsible for the antibacterial activity.

2. 2. 2. 3 Anti- Inflammatory Activity of Essential Oils:

Essential oils contain compounds that are known to possess strong anti-inflammatory properties, mainly terpenoids and flavonoids, which suppress the metabolism of inflammatory prostaglandins (Krishan and Narang, 2014). Also other compounds found in essential oils have anti-inflammatory, pain-relieving, or edema-reducing properties, for example linalool from lavender oil, or 1,8-cineole, the main component of Eucalyptus oil (Peana *et al*.,2003).

2. 2. 2. 4 Immunomodulatory Activity of Essential Oils:

Some essential oils positively influence the avian immune system, since they promote production of immunoglobulin, enhance lymphocytic activity, and boost interferon-γ release (Awaad *et al*., 2010; Faramarzi *et al*., 2013; Gopi *et al*., 2014; Krishan and Narang, 2014). Supplementing diets with essential oils containing herbal mixtures positively influenced the activity of the intestinal lymphatic system. The authors observed a reduced number of intraepithelial cells in the small intestine, which suggests possible relaxation of the strain resulting from the gastrointestinal defensive response. Essential oils are also used as immunomodulators during periods when birds are exposed to stress, acting protectively and re-generatively. Moreover, the oils alleviate the stress caused by vaccination (Barbour *et al*., 2011; Faramarzi *et al*., 2013; Gopi *et al*., 2014). In recent years studies have been carried out on the use of essential oils in conjunction with vaccination programs, including those against infectious bronchitis (IB), Newcastle disease, and Gumboro disease. The results of the experiments show that essential oils promote the production of antibodies, thus enhancing the efficacy of vaccination (Awaad *et al*., 2010; Barbour *et al*., 2011; Faramarzi *et al*., 2013).

2. 2. 3. Impact of Essential Oils on Nutrient Digestibility:

Several studies have shown antimicrobial properties of herb extracts which can improve intestinal micro flora population and enhance health in birds' digestive systems through reduction in number of disease-making bacteria (Mitsch *et al*., 2004; Jamroz *et al*., 2005). Intestinal health is of great importance in poultry for improved performance and feed conversion ratio. Nutrient absorption in the gastrointestinal tract is more effective with increase in the size and height of intestinal villi. Factors, such as diet were found to influence the morphology of the intestinal villi. In various domestic birds, there was a correlation between the morphology of the intestinal villi and food habits (Zulkifli *et al*., 2009). Phenolic compounds, such as carvacrol, thymol or eugenol, are used as additives in animal nutrition because of their antimicrobial activity.

2. 2. 4 Effects of Essential Oils in Serum Biochemistry:

Essential oils were observed to exert a hypocholesterolemic effect. The decrease in cholesterol levels may be due to an inhibition of the hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductive activity, which is a key regulatory enzyme in cholesterol synthesis (Hong *et al*., 2012). It is known that the absence or presence of cholesterolaemic effects of essential oils in an animal depend on breed, gender, age and the composition of the feed (Lee *et al*., 2003).

2. 2. 5 Effects of Essential Oils in Poultry:

Essential oils (EOs) represent a major group of phytogenic feed additives (PFA) (Zeng *et al*., 2015; Yang, 2015; Jacela *et al*., 2010). Due to their strong aromatic features and bioactivity, Essential Oils have been widely used since ancient times in aromatherapy, as flavor and fragrances in cosmetics and foods, and more recently as pharmaceuticals, natural preservatives, additives, and bio pesticides (Prakash *et al.*, 2012; Prakash *et al*., 2015; Pavela *et al.*, 2016). The bioactivity of Eos depends on their complex mixture of volatile molecules produced by the secondary metabolism of aromatic and medical plants (Prakash *et al.*, 2012; Ambrosio, 2017). Since ancient times, essential oils have been used due to their effects on humans and animals. They have been widely used for antibacterial, antiviral, fungicidal, insecticidal, acaricidal, antiparasitical, antipyretic, expectorant, anticancer, and cytotoxic activities (Bakkali, 2008 *et al.*; Edris, 2007; George *et al.*, 2009; Calo *et al.*, 2015). Essential oils have been studied for their ability to suppress the synthesis of mycotoxins, including aflatoxin (Dwivedy *et al.*, 2016). Furthermore, in poultry feeding, essential oils have roles as hypolipidemic and immune-modulating agents, as well as heat stress alleviators. Moreover, they are used as agents to reduce the methanogenesis rate in animal. (Gopi *et al*., 2013).

2. 2. 6 Uses of Essential Oils in Broiler Diets:

The essential oils as single or mixture may be used as a growth promoter in broiler production. Many studies have shown positive effects of dietary essential oil on body weight. Supplementing the dietary essential oils Cross *et al*., (2007); Bampidis *et al*., (2005) would stimulate the growth performance of broilers. The essential oils act as digestibility enhancer, optimizing the gut microbial ecosystem, stimulates the secretions of digestive enzymes and improves the growth performance in poultry (Lovkova *et al*., 2001; William and Losa., 2001; Cross *et al*., 2007). Ocak *et al.*, (2008) supplemented a mixture of herbal essential oils to broilers and found a significant reduction in feed intake which was also observed in the study performed by Lee *et al*., (2003), also who interpreted that essential oil and their mixture could positively affect the intestinal micro flora and thus digestion.

2. 3 Clove Essential Oils:

Clove (Eugenia ssp) considered as spices and appetizer. It contains 10% volatile oils which most of it eugenol, a substance that have an anesthetic effect (Mukhtar, 2011). Also, contain vitamins B and C, and the last vitamin involved in stress hormones synthesis thus has a major role in reducing body temperature by enhancing heat dissipation through blood vessels that are surrounding the body to maintain a relatively constant temperature (Khawala *et al*., 2012). In addition the clove contains phenols compounds that act as anti-bacterial agents.

2. 3. 1 Clove Essential Oils Properties:

Clove essential oil is colorless or pale yellow and darkens during storage. It is heavier than water and is characterized by persistent intense spicy scent and bitter, burning taste. Its composition is similar to the composition of the oil from the leaves. Eugenol and eugenol acetate are responsible for the smell of cloves (spicy, sweet with a hint of balsamic-fruit). Vanillin (deepening spicy character), caryophyllene and its derivatives (note of the wood and green) and trace amounts of cresols, guaiacol and methyl guaiacol (phenolic pungent odor) also affect the smell of clove oil (Isabel, 2009).

2. 3. 2 Composition of Clove Essential Oils:

Composition of clove oil altogether with its properties, depends on the origin of the plant, its growing season, the weather, time of day and air humidity. Another essential factor is the time which passes between the moment of picking up the raw material, and the production of oil. There are more than 100 components of clove oil worldwide (Gora *et al.*, 2005). The main component of clove oil - eugenol was identified by Bonastre in (1826). Eugenol in essential oil reaches from 30 to 95%, whereas eugenol acetate content amounts up to 22%. Oil from the growing leaves contains the smallest amount of eugenol (28%). The amount of eugenol increases to 95% as the leaves ripen, and content of eugenol acetate decreases from 51 to 1%. Eugenol and eugenol acetate Caryophyllene, Kopan, ilangen, humulen, kalamenen and heptane-2-one are present in smaller amounts in the oil. An important component of the oil, although present in small quantities, is vanillin aldehyde 4-hydroxy-3-methoxybenzoic, one of the most popular fragrance compounds. Vanillin is a crystalline substance in the form of colorless needles or crystalline powder, white to yellowish, with a melting point 80-81ºC and boiling point 284-285 ºC. Its strong aroma is perceptible at a concentration of

2x10-13 g/dm3 air. Both natural and synthetic vanillin is widely used in food industry. Vanillin can cause skin irritation especially among people with sensitive skin (Rutkowski *et al*., 2003; Jabłońska *et al*., 2008).

2. 3. 3 Uses of Clove Essential Oils:

The use of clove essential oil is now similar to those centuries ago. Historically, carnations, as a spice, used to help overcome indigestion. The oil, on the other hand, was used to lessen the toothache. Saint Hildegard wrote in her work Physical about cloves: “Who has the pain that roars in his head as if he were deaf, should often eat the cloves. Cloves are also recommended for toothaches (Gora *et al*., 2005). Their bactericidal activity was used the middle ages during the prevailing diseases. It was believed that cloves prevent contagion during epidemics of plague (Gora *et al*., 2005).

Nowadays the clove oil, an important natural antibacterial drug, is used in many fields, including dentistry, pharmaceuticals, and aromatherapy. It is used as an analgesic, anti-septic, warming, disinfectant, and antibacterial because it inhibits the growth or kills most pathogens, such as: E.scherichia coli, Mycobacterium phlei, Bacillus substilis, Streptococcus aureus, Aspergillus Niger, Penicillum chrysogenum. Oil is recommended for inhalation in the treatment of sore throat, colds, catarrh and inflammation of the mucous membranes of the mouth. It is also helps deal with any breathing problems, general weakness and neuralgia (Podlewski *et al*., 2010; Cimanga *et al*., 2002). Clove oil also has antioxidant effects (Gora et al., 2005). Aqueous and alcoholic extract of Turkish clove has been studied. Aqueous extracts oil at concentrations of 20, 40 and 60 g / ml showed inhibition of lipid per oxidation of linoleic acid in emulsion within the limits of 93.3-97.9%, while alcoholic extracts of the same concentrations shows inhibition between 94.9-98.2%. The obtained results make it possible to use cloves as a convenient and accessible source of natural antioxidants in food supplements and pharmaceutical preparations (Gora *et al*., 2005; Gulcin *et al*., 2004). Clove oil has anti-convulsant effects as well, which were already known in Persian folk medicine. The cloves were there used as a cure for epilepsy. This has been described by an Arab physician Avicenna who lived in the late tenth and eleventh century. Research was carried out to evaluate antistress activity of eugenol, the main component of clove oil (Pourgholami *et al*., 1999). Thanks to its characteristics, oil is an ingredient of many pharmaceutical preparations, ointments and painkillers. It is also a substrate for the production of dental analgesic preparations. In combination with zinc oxide is used to fill cavities in teeth. In addition, clove oil is included in mouthwash and gum liquids, toothpastes, and preparations for disinfection of hands. Eugenol is also widely used in dentistry. Zinc-eugenol paste is a good zinc-eugenol paste for the filling of dental canals in treating periodontitis. Furthermore, the paste acts antiseptic, local anesthetic and is resistant to moisture (Podlewski *et al*., 2010,)

Moreover, due to its activity the oil is widely used in aromatherapy. Massages and baths relieve various muscular and rheumatic pains, help with digestion problems, nausea and flatulence (Gora *et al*., 2005). However, clove oil can cause skin irritation and allergic reactions. It cannot be applied directly to the skin neither dissolved in vegetable oil nor in massage oil (Podlewski *et al*., 2010). It is also an important component of perfume products, especially those with an oriental flavor. It is also used for perfuming soaps (Gora *et al*., 2005). In aromatherapy there is a risk of undesirable side effects, such as irritation, contact allergy and phytotoxic reactions. Permissible concentration of the components (expressed in percent) of clove oil included in the products which are applied topically both in contact with human skin and removed from it are as follows (Janeczko *et al*., 2007): isoeugenol -0.02 and eugenol - 0.50. Clove oil boosts concentration and efficiency of thinking. In addition, revitalizes, energizes, exhibits analgesic and acts as an "aphrodisiac" (Jurkowska *et al*., 2005; Brud *et al*., 2009). Clove oil can also be used as an antioxidant and antimicrobial addition to oils, such as cotton oil. Addition of oil does not affect the color and appearance of cotton seed oil, also in concentrations of 50 - 1200 ppm does not change its smell (Park *et al*., 2009). Antifungal action of clove oil and eugenol were also used in the food industry. Products may be contaminated with microorganisms during harvesting, technological process and storage of food. Since ancient times, in order to inhibit the growth of fungi gaped and spices were used, they also including cloves (Maryam *et al*., 2007). Effects of oil on Aspergillus flavus (a kind of widespread fungi) which can produce potentially carcinogenic aflatoxin, has been studied. The medium was the tomato puree. Studies have shown that the use of oil in quantities necessary to achieve the antifungal effect has a bad influence on the organoleptic properties of tomato puree (taste). This disadvantage can be avoided by applying oil to products with a strong taste that camouflage a clove flavor or adding only the active ingredients of essential oil (Stępień *et al*., 2007). Fungus Penicillium Citrinum can produce mycotoxins (citrinin) causing kidney damage and bleeding (Vazquez *et al*., 2001). Citrate may appear in dairy products. Therefore, oil and eugenol was tested as an inhibitor of the development of Penicillium citrinum, during the production of regional cheese manufactured in the Spanish province of Galicia from un-pasteurized milk in the presence of animal rennet (Venarsky *et al*., 2006). Isolated from the essential components of the so-called isolates are also used in the food industry. Anesthetic properties of clove oil may also find practical use as a substance blocking and reducing stress and pain of living organisms such as fish during their studies (including the measurement of length and weight). The use of oil in such studies is effective. There was no mortality of fish observed. In addition, oil is a local and not expensive product in countries such as Indonesia. For studies of coral reefs essential oil concentration should be carefully selected and solvent should be also reasonably used, since they may have a negative impact on corals (e.g. Pocillopora verrucosa) - fading, inhibition of growth. The measures used in biological research should be characterized by rapid and short duration of action of the body to return to the normal state. Furthermore, it should be characterized by lack of toxicity of both, the test organism and the investigator, no adverse effects with repeated use, ease of use and low price (Makame. 2008). Clove oil is also used for production of deodorizing and preparations acting as a deterrent for mosquitoes (Gora *et al*., 2005; Podlewski J.K., 2010). Another area in which essential oils were applied (including clove) is painting. They are used here to dilute the paint, as a binder in oil painting (Makame. 2008). Studies on the clove oil with stalks as fuel additive for diesel engines were interesting. Oil decreased the ignition temperature of fuel, which allows for its safe storage and transport, but such modifications of fuel, due to the price of oil, are not profitable (Makame. 2008).

2. 3. 4 Effects of Clove Essential Oil in Poultry Performance:

Eugenol is a major component of clove extract and exhibits a wide range of anti-microbial activity in vitro (Ehrich *et al*., 1995). Clove, and its essential oil, is one of the plant extracts that has been found effective in poultry to improve growth performance, control some intestinal pathogens, acts anti-septic and as digestion stimulant, and shows strong anti-microbial and anti-fungal, anti-inflammatory, anesthetic, anti-carcinogenic, anti-phrastic and anti-oxidant effects (Najafi and Torki., 2010; Mitsch *et al*., 2004; Kamel., 2001; Ehrich *et al*., 1995). Cinnamaldehyde and eugenol have been reported to possess anti-bacterial activity against a wide range of bacteria and inhibitory properties against Aspergillus flavus (Steiner, 2010; Toghyani *et al*., 2011). Therefore, the present study was conducted to evaluate the effect of adding graded levels of mixed essential oils, clove essential oil with other essential oils on growth performance and blood parameters in broiler chickens.

2. 3. 5 Effects of Clove Essential Oil on Serum Constituents:

In the present study, dietary clove essential oil mixed with others and supplementation has not affected serum levels of cholesterol, triglyceride, HDL, LDL and. Blood glucose was affected by the treatments, so blood glucose levels were reduced in clove essential oil-containing treatments compared to control treatment. Insulin-like biological activity of clove and other culinary or medicinal plant aqueous extracts were also proven in vitro. Various mechanisms for a hypoglycemic effect have been proposed and many phenolic compounds isolated from medicinal plants can significantly produce these effects (Broadhurst *et al*., 2000; Baker *et al*., 2008) reported the use of medicinal plants by stimulating insulin secretion and prevent cellular resistance to insulin, reduced blood glucose levels, microbial population, gut micro flora has significant effects on host nutrition, health and growth performance by interacting with utilization and development of the gut system of the host. This interaction is very complex and depends on the composition and activity of the gut micro flora. It can have either positive or negative effects on the health and growth of birds (Giannenasa *et al*., 2012). Generally, the essential oils possesses the strongest anti-bacterial properties against pathogens. Essential oil is containing a high percentage of phenolic compounds such as carvacrol, eugenol and thymol (Brenes and Roura, 2010).

Improvements in treatments containing clove essential oil are probably due to the clove oil anti-bacterial properties. Eugenol had good anti-microbial activity against some bacterial species such as Listeria monocytogenes and Campylobacter jejuni, but had medium anti-microbial activity against Enterobacteria (Giannenasa *et al*., 2012). Also, Tucker, (2002) reported that blend of essential oils increased numbers of lactobacillus spp. in broilers. And, Dalkilic *et al*., (2009) showed that the clove extract and antibiotic supplementation decreased total coliform microorganism counts of small intestine of broilers at 21 and 42 days of age of the experiment. Rodriguez-Vaquero *et al*., (2007) demonstrated the anti-microbial properties of phenolic compounds against Escherichia coli. Small intestinal morphology the gastrointestinal mucosa is the first tissue that comes in contact with dietary constituents. Mucosa status and their microscopic structure can be good indicators of the response of the intestinal tract to active substances in feeds. Changes in intestinal morphology, such as shorter villi and deeper crypts, have been associated with the presence of toxins (Viveros *et al*., 2011). There is only slight evidence of active plant oils on morphological and histological characteristics in animals fed on diets supplemented with plant extracts.

2. 4 Basil Essential Oils:

Basil (Ocimum basilicum L.) is an annual plant of the Lamiaceae family, growing wild in subtropical and tropical areas of America, Africa, Asia, and in some southern regions in Europe (Kwee *et al*., 2011). Today, basil belongs to worldwide cultivated aromatic plants. The cultivation of basil is performed under natural as well as greenhouse conditions. To increase the yield and to produce basil year round, cultivation in a greenhouse is more suitable than cultivation in an open field (Sgherri *et al*., 2010). Furthermore, in comparison to traditional soil culture, hydroponic cultivation of basil has additional benefits, such as using less ground area to obtain a higher yield of biomass characterized by better quality properties (Kiferle *et al*., 2011).

Traditionally, basil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms, and kidney malfunction (Kaurinovic *et al*., 2011). The medicinal properties of basil are associated with the presence in its leaves of a whole complex of biologically active compounds of various chemical structures (Singletary, 2018). In particular, it has been found that basil leaves are rich in phenolic acids (rosmarinic, chicoric, caffeic, and caftaric) (Flanigan *et al*., 2014), flavonol (quercetin, kaempferol) glycosides and anthocyanins (Złotek *et al*., 2016; Ghasemzadeh *et al*., 2016). The phenolic compounds listed above make the main contribution to the anti-oxidant properties of basil leaf extracts. Another important component of basil leaves and flowers is essential oil, which is of high value for the food and pharmaceutical application of this plant. The essential oils distilled from various basil cultivars can contain linalool, methyl chavicol, 1, 8 -cineole, eugenol, methyl eugenol, methyl isoeugenol, thymol, methyl cinnamate, citral, and camphor (Avetisyan *et al*., 2017). In several studies the anti-oxidant, anti-microbial, anti-inflammatory, anti-bacterial, anti-fungal activities as well as repellent, insecticidal, larvicidal and nematicidal activities of basil essential oils have been established (Avetisyan *et al*., 2017; Kavoosi *et al*., 2017; Saggiorato *et al*., 2012).

2. 4. 1 Properties of Basil Essential Oils:

The Ocimum genus belonging to the Lamiaceae family Omer *et al*., (2008), includes approximately 150 species Javanmardi *et al*., (2002), with a great variation in phenotype, oil content, composition, and possibly bioactivity (Simon *et al*., 1999). Ocimum sanctum L. and Ocimum basilicum L. are the two basil species that are considered to be promising essential oil crops. The basil essential oil contains pleasant aroma and is known to possess anti-microbial, anti-oxidant Bozin *et al*., (2006); Suppakul *et al*., (2003) and insecticidal Aslan *et al*., (2004) activities. Basil essential oil is a major aromatic agent with applications in various industries, such as the food, pharmaceutical, cosmetic, and aromatherapy industries (Trevisan *et al*., 2006). There is a great variation of essential oil composition (and aroma) among basil cultivars currently on the international market.

2. 4. 2 Composition of Basil Essential Oils:

Basil has a complicated taxonomy due to the numerous varieties of cultivars within the species that do not differ significantly in morphology. Thus, the classification of genotypes only by morphological features becomes difficult due to anthropogenic interference with selection, cultivation, and hybridization (Valls, 2007). Due to the hybridization of several species and varieties, there is a wide variability of the chemical constituents (Simon *et al*., 1990). Chemical characterization can be used to separate the accessions based on the presence or concentration of specific substances and to determine the intrinsic variability or variability among accessions of the same species (Valls, 2007). Despite the wide variation in the chemical composition of basil essential oil within the same species, monoterpenes and phenylpropanoids predominate (Tateo., 1989; Marotti *et al*., 1996). Genotype characterization based on the chemical constitution of the essential oil has been used in several cultures such as Zingiber officinale Wohlmuth *et al*., (2006) and Hyptis suaveolens Azevedo *et al*., (2002), including plants of the genus Ocimum, with emphasis on O. basilicum (Telci *et al*., 2006). Several studies assessing the chemical composition of 18 basil essential oils observed that the samples distributed into seven distinct types, each one presenting as the major volatile compound among the following: linalool, methyl cinnamate, methyl cinnamate/linalool, methyl eugenol, citral, methyl chavicol (estragole), and methyl chavicol/citral. A total of 27 basil cultivars were characterized according to the chemical composition of their essential oils, and the cultivars were grouped into five different types: eugenol/linalool (30–35% linalool and 12–20% eugenol); linalool (52–66%); estragole/linalool (22–38% estragole and 21–37% linalool); *(Z)* methyl cinnamate (19–38%); and estragole (38–95%) (Liber *et al*., 2011). The chemical characterization of 38 basil genotypes resulted in seven groups: linalool (19–73%); linalool/eugenol (28–66% linalool and 5–29% eugenol); methyl chavicol (20–72% methyl chavicol); methyl chavicol/linalool (8–29% methyl chavicol and 8–53% linalool); methyl eugenol/linalool (two accessions with 37% and 91% methyl eugenol and 60% and 15% linalool); methyl cinnamate/linalool (9.7% methyl cinnamate and 31% linalool); and bergamotene (one accession with bergamotene as the major constituent) (Zheljazkov *et al*., 2008).

2. 4. 3 Uses of Basil Essential Oils:

Basil seeds improved productive performance of broiler chicks and decreased serum cholesterol (Rabia., 2010).The leaves and flowering tops of sweet basil are used as a carminative, galactogogue, stomachic and anti-spasmodic in folk medicine (Sajjadi., 2006). However, recently the potential uses of basil essential oils, particularly as anti-microbial and anti-oxidant agents, have also been investigated. There is extensive diversity in the constituents of the basil oils, and several chemo types have been established from various phytochemical investigations (Sajjadi, 2006). However, methyl chavicol, linalool, methyl cinnamate, methyl eugenol, eugenol and geraniol are reported as major components of the oils of different chemotypes of O. basilicum (Sajjadi, 2006). Many research strategies have been practiced particularly introducing feed supplements and feed additives in poultry feds (Christaki and Bonos *et al.*, 2012). Nweze and Ekwe., (2012) concluded that Ocimum leaf extracts can be used to improve growth performance, stabilize the blood components and reduce the gut and blood micro-organisms for finishing broilers. Utilization of basil leaf in livestock nutrition has not been widely and scientifically exploited. The use of basil leaf in growing pullets will increase the knowledge for the search for natural herd and reduce cost of production.

2. 5 Eucalyptus Essential Oils:

Eucalyptus is a medicinal plant that belongs to Myrtaceae family, originated in Australia but found worldwide, especially in tropical and subtropical regions (Salari *et al*., 2006). It contains several vital compounds including p-cymene, 1, 8-cineole, β-phellandrene, spathulenol, cryptone aldehydes, cuminal, uncommon and phellandral, α-phellandrene, β-phellandrene leading to multi-functional characteristics such as anti-microbial, anti-inflammatory and anti-oxidative properties (Bokaeian *et al*., 2010). In humans, eucalyptus essential oils is used to reduce nasal congestion in common cold during cold winter months (Sadlon and Lamson, 2010). On pathogenic bacteria isolated from specimens of patients with respiratory tract disorders. Supplementing diet with 0.1% eucalyptus essential oil can increase live body weight gain and growth rate in broiler chicks (Osman *et al*., 2007). Furthermore, polyphenols in eucalyptus leaves have shown various biological activities including anti-oxidant activity, anti-tumor activity and anti-bacterial activity (Salari *et al*., 2006; Bokaeian *et al*., 2010; Chen *et al*., 2017). Whereas Ahmed *et al*., (2005) reported that dietary eucalyptus essential oil had no significant effect on live body weight and daily weight gain in growing rabbits. Although many aspects of bioactive properties of eucalyptus essential oil have been explored in humans.

2. 5. 1 Properties of Eucalyptus Essential Oils:

Eucalyptus is an important ethno- medicinal plant belonging to the family of myrtaceae Mohammed *et al*., (2012), it is used for food and eucalyptol (1, 8-cineole) is listed as a synthetic flavoring agent Barnes *et al*., (2002), color, aroma and preservation of food or beverages (Burt, 2004). The pharmacological studies revealed that Eucalypts possessed gastro intestinally, anti-inflammatory, anti-oxidant, anti-microbial (Al-Snafi., 2017). Medicinal eucalyptus oil is widely used for the relief of cold and influenza symptoms. It is a unique natural product having anti-septic properties and the power to clear the nasal passages and bronchial tubes making it easier to breathe (Hasegawa *et al*., 2008).

2. 5. 2 Composition of Eucalyptus Essential Oils:

Eucalyptus essential oil is the generic name for distilled oil from leaf of eucalyptus. The leaves of eucalyptus globules contain up to 3.5 ww essential oil (Melka *et al*., 2010). 1, 8- cineole eucaptol is the principal constituents found in eucalyptus, however other chemo types such as a- phella, a-phellandrene, p- cymene, y- terpinene, ethanone, spathulend, among others have been documented (Akin *et al*., 2010; Chalchat *et al*., 2001; Igbal *et al*., 2011). Also Khaled *et al*., (2015) identified the major constituents in the eucalyptus leaf essential oils as 81.41 monoterpene hydrocarbons, 12.55 were oxygenated monoterpenes and it also contained 0.5 sesquiterpene hydrocarbons. Slavenko *et al*., (2010) analyzed the chemical composition of the leaf essential oil. They found that monoterpene hydrocarbons were the major class of compounds. Among them, dominant compounds were p-cymene 17.38-28.6, pallendrene 12.35- 14.47 and pinine 0.94-11.48. Eucalyptus camaldulensis leaves contained 0.98 and 0.96 essential oils from saline and non-saline areas respectively. The principal constituents in the essential oils from saline and non-saline was 1, 8 cineole, 34.42 and 40.05, a-pinene 14.68 and 12.43, y-terpinen 9.42 and 7.48, ledol 7.42 and 7.67 and t-pinocarvol 8.36 and 3.32, respectively (Muhammad *et al*., 2010).

2. 5. 3 Uses of Eucalyptus Essential Oils in Poultry:

Carli *et al*., (2008); Awaad *et al*., (2010) and Rehman *et al*., (2013) reported that eucalyptus essential oil is a promising alternative to improve thermal comfort to chickens, as well as their immune responses. This oil has positive effects in reducing thermal sensation in birds, promoting freshness in thermoregulatory sensors of the endocrine system, being an alternative to reduce the deleterious effects of heat on broilers (Awaad *et al*., 2010). Its essential oil has active molecules such as menthol and 1.8-cineol that can generate the feeling of freshness, stimulation of the immune system, inhibition of inflammatory responses, and anti-microbial effect (Rehman *et al*., 2013). Awaad *et al*., (2010) and Rehman *et al*., (2013) highlighted the anti-microbial and immunostimulatory effects of eucalyptus essential oil on the respiratory tract of birds, enhancing immune responses, and reducing the incidence of mucosal aggression in the respiratory tract of chickens (Cermelli *et al*., 2008). The immune system stimulation effect occurs due to the anti-inflammatory effect Santos and Rao, (2000); Juergens *et al*., (2003); Greiner *et al*., (2013);

Lima *et al*., (2013) and the stimulation of phagocytosis Serafino *et al*., (2008); Yadav and Chandra, (2017) caused by 1.8-cineol in the organism.

CHAPTER THREE

MATERIALS AND METHODS

Four experiments were conducted to evaluate different sources of mixed essential oils: Eucalyptus oil, Clove oil, Basil oil in combination of them (1:1:1) at graded levels in broiler rations, at College of Agricultural Studies, Sudan University of Science and Technology.

3.1 Experiment (1):

Response of broiler chicks to graded levels of Eucalyptus and Clove mixed essential oils at (200, 400, and 600) mg/kg:

This experiment was conducted during winter season from (19 of January to 23 February 2019).The ambient temperature average 20–26 c^{o} (appendix 1) during the experimental period (5 weeks).

3.1.1 Experimental Chicks

A total number of (96) one day old commercial un-sexed broilers of Cobb strain were obtained from (Dajin Breeder Company (Mico) - Sudan) and transported to student poultry premises, (Shambat). The chicks were adaptedfor one week to the premises and fed on pre-starter before the start of experimental period. At the end of adaptation period, all chicks were weighed with an average initial weight of (185 g). The chicks were then distributed randomly into four experimental groups A, B, C and D with three replicates per each and with eight chicks' arrangement (4x3x8) in a complete randomized design (CRD), feed and water provided *ad libitum* through-out the experimental period. Chicks were bought vaccinated against Newcastle disease (ND) and against Infectious Bronchitis disease (IBD) in the hatchery by (ND+IB) spray on day one, inactivated ND injection and Gumbobest injection day one. On farm vaccinated against Gumboro disease by Bur 706-France at (11) days of age, and against New-castle disease by Avinew –France at (18) days of age. The dosage was repeated at (22) and (28) days of age for Gumboro disease by Bur (706 – France) and for ND by Avinew – France respectively.

Combinations of AD3 (pantominovite – pantexHoland and B.V. 5525 ZG DuizelHoland), As a soluble multi- vitamins was provided three days before and after vaccination programs in order to guard stress.

3.1.2 Housing

The experimental house in which the chicks were kept was semi closed, located in east –west direction. The housing dimensions were 25 m. length, 8.8 m. width and 3.05 m height. The roof ceiling was made of trapezoid corrugated aluminum sheet and was insulated of (100 mm) glass wool with thermal conductivity of (0.04 w/m^2). The walls of the house on the northern and southern sides were built from red blocks raised high to the level of 0.69 m. the house was equipped with adjustable side wall curtains to control the flow of air inside house. The top and bottom of the curtain opening was equipped with a curtain rod to minimized draft when closed. The floor was tightly concreted.

Mechanical ventilation system was used in the house to generate on one direction air flow to provide the required levels of uniformity of air distribution over wide range of climatic condition. The house have two exhaust fan (fan diameter 1.29 with air 44500 m^2/h). Positioned in the middle of the western side wall, were to maintain negative pressure inside the house as a result of negative pressure outside air flows into the house through inlet opening with cellulose pad besides maintaining the desired temperature and ventilations inside also an outlet on the roof was required to exit surplus heat, gases, moisture and supply fresh air.

The cooling pad banks dimensions were (4 m. long × 1.4 m. length × 0.15 width) and that of air inlet valve was 0.45 m. the cooling pad was situated at two sides, north and south direction at the rear of the poultry house.

Cooling pad was made of specially impregnated cellulose paper of wait ability, arranged in self- supporting structure that guaranteed long life without any sagging.

The other integral components provided with each pad cooling bank were pump, polyester, water tank capacity (1000 liters), for storage of water which was continuously supplied from main tap water under control of flouter which was put in the tank. Also there was one horse power electrical motor for pumping water from the tank to the top of pad cooling banks. There was piping system for supply and return of water, the cooling and humidification of outside air is obtain by evaporation of very fine water particles. Due to negative pressure maintained by the exhaust fans air flow through the pad and then through special air inlet to the house.Special design of the pads enables the air to pass through small opening or flutes in furious state, thus creating ideal condition for maximum evaporation and consequently maximum cooling to take place as a result of the layer contact area between water and air, excess water is returned to the bank where it is pumped to the top edge of the pad for re-circulation.

Twelve cages experiments (1.5 × 1 m.) were prepared using wire mesh portioned and then were cleaned washed and disinfected by formalin and white phenol solution.

Before start the experiment, a layer of wood shairy (5cm) thickness was laid on the floor as littler material. Each cage was provided by (5 kg) rounded feeder and (2.5 lit.) baby drinker which were adjusted to the progressive growth of chicks.

The light program was 24 hours light from 1-3 days and 23 hours day for the rest of the period.

3.1.3 Experimental Rations:

Air-dried eucalyptus leaves and clove fruits were submitted to hydro distribution using n-hexane as a collecting solvent. The solvent was removed under vacuum and the quantities of the essential oils were determined by gas chromatography, at Industrial Research Center, Khartoum North. And the chemical composition of the essential oils were determined by GC- MS analysis, at Regional Center for Food and Feed, Ministry of Agriculture, Giza, Egyptian Arabic Republic. The chicks were divided into four dietary treatments (A, B, C and D), the first group (A) fed on control diet (without essential oil), the other groups (B, C and D) were fed on the based diet supplemented with eucalyptus and clove mixed essential oils (1;1) as growth promoter, at levels of 200 , 400 , 600 mg/kg feed respectively. The control diet was created to meet the nutrient requirements of the broilers according to Nutritional Research Council (NRC. 1994).

The ingredients percent as a composition were calculated and the chemical composition of the experimental control diet were presented in tables (1, 2 and 3). Experimental diets were fed for five weeks.

Table (1): Composition of control diet ingredients

Ingredients	**%**
Dura	64.289
Ground nut cake	12.000
Sesame cake	17.000
Broiler concentrate*	5.000
Di-calcium phosphate	0.618
Oyster shell	0.487
Lysine	0.243
Methionine	0.113
Salt	0.25
Total	100

*****crude protein 40; crud fiber; calcium; phosphorus (aval) lysine methionine met +sys 3.20; met energy 2100 k cal; Sodium 2.60. Product vits. A: 200 mg/Kg; vit E: 500 mg/kg; vit B1: 15 mg/kg; vit B2: 100 mg/kg; vit B6: 20 mg/kg ;vit B12: 300 mg /kg; Biotin:1.000 mg/kg; Nicotinic acid: 600 mg/kg; Folic acid: 10 mg/kg; vit K: 30 mg/kg; pantothenic acid: 150 mg/kg; choline chloride: 5.000 mg/kg; copper: 100 mg/kg; iodine: 15 mg/kg; Cobalt: 3 mg/kg; selenium: 2 mg/kg; manganese: 1200 mg/kg; zinc: 800 mg/kg; iron: 1.000 mg/kg; B.H.T: 900 mg/kg; Salinomycin-Na: 1.200 mg/kg .

Table (2): Calculated composition of experimental control diet

ME/Kcal	3111.o26
Nitrogen Free Extract	58.86
Crude protein	22.802
Crude fiber	4.o99
Lysine	1.393
Methionine	0.597
Calcium	1.176
Phosphor	0.766

Table (3): Chemical composition of control diet

Dry Matter %	94.00
Moisture %	6.00
Ash %	4.60
Crude Protein %	23.19
Crude Fiber %	4.35
Ether Extract %	3.00

3.1.4 Data Collected:

3.1.4.1 Performance Data:

Average body weight and feed consumption (g) for each group were determined weekly through-out the experimental period. Body weight gain and feed conversion ratio (FCR) were calculated weekly. Health of the experimental herd was closely observed and the mortality recorded daily.

3.1.4.2 Slaughter procedure:

At the end of the experimental period (5weeks) birds were fasted overnight with only water allowed. Three birds of similar live body weight were selected randomly from each treatment group and weighed individually before slaughter by severing the right and left carotid and jugular vessels, trachea and esophagus. After bleeding they were immerged in hot water, hand plucked and washed. Head was removed closed to skull, feet and shanks were removed at the hock joint. Evisceration was accomplished by posterior ventral cut to completely remove the visceral organs (heart, liver, gizzard, abdominal fat and intestine) and then were separated weighed individually and were expressed as a percentage of live weight. The hot carcasses were weighed to calculate the dressing percentage.

3.1.4.3 Carcass Characteristics:

The hot carcass was prepared for analysis by removal of the skin and neck near to the body and each was weighed separately. The carcass was then divided into two parts right and left sides by mid sawing along the vertebral column and each side was weighed. The left side was divided into three commercial cuts: breast, thigh, and drumstick, each cut was weighed separately, and were expressed as percentage of the carcass weight. Then they were deboned, the meat and bone were weighed separately, and were expressed as percentage of their cuts. The meat was frozen and stored for meat analysis.

3.1.4.4 Blood Serum Profile:

Blood samples withdrawal from jugular veins. Serum prepared from the blood analyzed for concentration of metabolites total protein, albumin, cholesterol, cholesterol HDL, cholesterol LDL, triglycerides, glucose, urea, uric acid, creatinine, enzyme activities ALP, AST and minerals (Ca, P).

3. 2 Experiment (2):

Response of broiler chicks to graded levels of Eucalyptus and Basil mixed essential oils (200, 400, and 600) mg/kg:

3. 2. 1 Experimental Chicks

A total number of (96) one day old commercial un-sexed broilers chicks of Cobb strain were obtained from the same company of the first experiment. Procedures were typical to those mentioned in the first experiment too.

3. 2. 2 Housing

The housing in this experiment is the same as that mentioned in the first one, and the same procedures too.

3. 2. 3 Experimental Ration

Eucalyptus leaves and Basil plants were obtained from the scattered trees and bushes in the streets around, dried, then submitted to the oil extraction at Industrial Research Centre. Khartoum North by the method of hydro distribution. And the chemical composition of the essential oils were determined by GC- MS analysis, at Regional Center for Food and Feed, Ministry of Agriculture, Giza, Egyptian Arabic Republic. Four experimental diets (A, B, C and D) were formulated. The chicks were divided to four experimental diets. Group A chicks was fed on control diet. Group B, C and D were fed on the control diet supplemented with (200, 400, 600) mg/kg eucalyptus and basil mixed essential oils (1: 1) as successive.

To meet the feed requirements percentage of the broilers, the control diet was created according to Nutritional Research Council (NRC, 1994).

3. 2. 4 Data Collected

It was the same data collected that mentioned in the first experiment. Performance data, slaughter and carcass procedure, blood serum, enzyme activities, minerals and metabolic indicator.

3. 3 Experiment (3):

Response of broiler chicks to graded levels of Clove and Basil mixed essential oils at (200, 400, and 600) mg/kg:

3. 3. 1 Experimental Chicks

A total number of (96) one day old commercial un-sexed broiler chicks of Cobb strain were obtained from the same company of the first experiment. Procedures were typical to those mentioned in the first one too.

3. 3. 2 Housing

The housing here is the same one that mentioned in the first experiment, and the same procedures too.

3. 3. 3 Experimental Ration

Clove fruits were obtained from the market, Clove fruits and basil plants were dried, then submitted to the oil extraction at Industrial Research Center, Khartoum North by the method of hydro distribution. And the chemical composition of the essential oils were determined by GC- MS analysis, at Regional Center for Food and Feed, Ministry of Agriculture, Giza, Egyptian

Arabic Republic. Four experimental diets (A, B, C and D) were created according to National Research Center (NCR, 1994), to meet feed requirements of the broiler chicks.

3. 3. 4 Data Collected

It was the same data collected that mentioned in the first experiment. Performance data, slaughter and carcass procedure, blood serum, enzyme activities, minerals and metabolic indicator.

3. 4 Experiment (4):

Response of Broiler chicks to graded levels of Eucalyptus, Clove and Basil mixed essential oils at (200, 400, and 600) mg/kg:

3. 4. 1 Experimental Chicks

A total number of (96) one day old commercial un-sexed broiler chicks of Cobb strain were obtained from the same company of the first experiment. Procedures were typical to those mentioned in the first experiment too.

3. 4. 2 Housing:

The housing in this experiment is the same one that mentioned in the first experiment, and the same procedures too.

3. 4. 3 Experimental Ration:

Combination of Eucalyptus with Clove with Basil mixed essential oils was created by (1: 1: 1) ratio.

Four experimental rations were created. In groupA chicks were fed on the control diet, same as the above experiments. In group B, C and D chicks were fed on the control diet with 200, 400 and 600 mg/kg combination of essential oils as successive.

3. 4. 4 Data Collected

It was the same data collected that mentioned in the experiment one. Performance data, slaughter and carcass procedure, blood serum, enzyme activities, minerals and indications of metabolic.

3. 5 Methods:

3. 5. 1 Method of oils extraction

Eucalyptus leaves, Clove fruits and Basil plants were extracted at Industrial Research Centre Khartoum North, to obtain the essential oils from each of them.

The oil extraction machine is composed of three parts: The first is the evaporative part, next the condensing part and the last one is the separating oil part. Water poured in a cylinder shape in the lower part, the plants part (leaves, fruits, plants) in the upper part over the water in the cylinder with a barrier between them to allow evaporation of steam throw it upward to the plants tissues which contains the volatile oil, beneath the cylinder there is source of heat (oven). The water boiled and the steam evaporated upward throw the plant with water and oil to upper tube in a cool place to condense in the condenser, the steam changed to a liquid form and enter throw another tube to the last part and poured in a beaker, water and oil mixed with each other. The density of oil is lighter than water, so oil floats in the upper and water remain in the lower. The water is flushed out side by the lower opening under the beaker, then oil is collected.

3. 5. 2 Chemical Methods:

3. 5. 2. 1 Serum Determination:

Blood from the venous obtained from the chicks at the end of the experiment without heparin, and centrifuged at 3000 r. p. m for 5 minutes, then serum was stored at 20ºc until analyzed in the National Public Health Laboratory (STAK), Chemical Pathology Department. Using Bio system (A) 25, made in Germany. Quality system certified according to EN ISO 13485 and EN ISO 9001 standards.

Procedure of system:

Full automated biochemical analyzer.

Well prepared sample and reagent.

Well calibrated and controlled analyzer.

Insert samples and code number.

Select tests and click on the position in the bottom.

Better to use test tube rather than cubs.

Click on accept and then click start.

Analyze by batch not by individual sample.

For result click on current state (result) then print.

Reagents preparation:

Reagents are provided ready to use for measurements of serum samples, kits provided by Bio Systems S.A. Costa Brava, 30.08030 Barcelona (Spain).

3. 5. 2. 1. 1 Aspartate Amino Transferase (Glutamyl Oxaloacetic Transaminase) (AST/GOT)

Principle of the method:

Aspartate aminotransferase (AST or GOT) catalyzes the transfer of the amino group from aspartate to 2-oxglutarate, forming oxalacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the malate dehydrogenase (MDH), coupled reaction[1, 2, 3, 4].

$$\text{Aspartate + 2-Oxoglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate + Glutamate}$$

$$\text{Oxaloacetate + NADH + } H^+ \xrightarrow{\text{MDH}} \text{Malate + } NAD^+$$

Composition:

A. Reagent: 5 x 40 mL.Tris 121 mmol/L, L-aspartate 362 mmol/L, malate dehydrogenase > 460 U/L, lactate dehydrogenase > 660 U/L, pH 7.8.
   WARNING: H315: Causes skin irritation. H319: Causes serious eye irritation. P280: Wear protective gloves/protective clothing/eye protection/face protection. P305+P351+338: IF IN
   EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P332+P313: If skin irritation occurs: Get medical advice/attention.

B. Reagent: 5 x 10 ml. NADH 1.9 mmol/L, 2-oxoglutarate 75 mmol/L, sodium hydroxide 148 mmol/L, sodium azide 9.5 g/L.
   WARNING: H302: Harmful if swallowed. EUH031: Contact with acids liberates toxic gas. P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. P330: Rinse mouth.

Storage:

Store at 2-8 ºC.

Reagents are stable until the expiry date show on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

-Reagents: Presence of particulate material, turbidity and absorbance of the blank lower the limit indicated in "Assay parameters".

3. 5. 2. 1. 2 Alkaline Phosphatase (ALP) - AMP 2-Amino-2-Methyl -1-Propanol Buffer:

Principle of the method:

Alkaline phosphatase (ALP) catalyzes in alkaline medium the transfer of the phosphate group from 4-nitrophenylphosphate to 2-amino-2-methyl-1-propanol (AMP), liberating 4-nitrophenol. The catalytic concentration is determined from the rate of 4-nitrophenol formation, measured at 405 nm[1].

$$\text{4-Nitrophenylphosphate} + \text{AMP} \xrightarrow{\text{ALP}} \text{AMP–phosphate} + \text{4-Nitrophenol}$$

Composition:

A. Reagent: 2-Amino-2-methyl-1-propanol 0.4 mol/L, zinc sulfate 1.2 mmol/L, N-hydroxy-ethyl-ethyl-enediaminetriacetic acid 2.5 mmol/L, magnesium acetate 2.5 mmol/L, pH 10.4.

B. Reagent 4-Nitrphenylphosphate 60 mmol/L.

Storage:

Store at 2-8 ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during use.

Indications of deterioration:

-Reagents: Presence of particulate material, turbidity, absorbance of the blank over 1.200 at 405 nm (1 cm cuvette).

3. 5. 2. 1. 3 Total Protein:

Protein in the sample reacts with Copper (II) ion in alkaline medium forming a colored complex that can be measured by spectrophotometry[1].

Composition:

A. Reagent, Copper (II) acetate 6 mmol/L, potassium iodide 12 mmol/L, sodium hydroxide 1.15 mol/L, detergent.

Corrosive (C): R34: Causes burns. S26-45: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. In case of accident or if you feel unwell, seek medical advice immediately.

S. Protein Standard. Bovine albumin. Concentration is given on the label. Concentration value is traceable to the Standard Reference Material 927 (National Institute of Standards and Technology. USA).

Storage:

Reagent (A): Store at 15-30 ºC.

Protein standard (S): Store at 2-8 ºC, once opened.

Reagent and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

-Reagent: Presence of particulate material, turbidity, absorbance of the blank over 0.150 at 545 nm.

-Standard: Presence of particulate material, turbidity.

3. 5. 2. 1. 4 Cholesterol – Cholesterol Oxidase/Peroxidase:

Principle of the method:

Free and esterified cholesterol in the sample originates, by means of the coupled reactions described below, a colored complex that can be measured by spectrophotometry[1,2].

Cholesterol ester +H_2O $\xrightarrow{\text{Chol.esterase}}$ Cholestero1+ fatty acid

Cholesterol +$^1/_2O_2$ + H_2O $\xrightarrow{\text{Chol.oxidase}}$ Cholestenone+H_2O_2

$2H_2O_2$ + 4-Amino antipyrine + phenol $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + 4 H_2O

Composition:

A. Reagent. 10 x 50 ml. Pipes 35 mmol/L, sodium cholate 0.1 U/ml, peroxidase > 0.8U/ml, 4-aminoantipyrine 0.5 mmol/L, pH 7.0.

Storage:

Store at 2-8 ºC. Reagent is stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use. Indications of deterioration:

-Reagent: Presence of particulate material, turbidity, absorbance of the blank over the limit indicated in "Assay parameters".

3. 5. 2. 1. 5 Urea /Bun-UV (Urease/Glutamate Dehydrogenase):

Principle of the method:

Urea in the sample consumes, by means of the coupled reactions described below, NADH that can be measured by spectrophotometry[1,2].

Urea + H_2O $\xrightarrow{\text{urease}}$ $2NH_4^+$ CO_2

NH_4^+ + NADH + H^+ + 2-oxoglutarate $\xrightarrow{\text{Glutamate dehydrogenase}}$ Glutamate + NAD^+

Composition:

A. Reagent: 5 x 40 mL Tris 100 mmol/L, 2-oxoglutarate 5.6 mmol/L, urease > 140 U/mL, glutamate dehydrogenase > 140 U/mL, ethyleneglicol 220 g/L, sodium azide 0.95, pH 8.0.

WARNING: H302: Harmful if swallowed. P301 + P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. P330: Rinse mouth.

B. Reagent: 5 x 10 mL, NADH 1.5 mmol/L, sodium azide 9.5 g/L.

WARNING: H302: Harmful if swallowed. EUH031: Contact with acids liberates toxic gas. P301 + P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. P330: Rinse mouth.

Storage:

Store at 2-8 ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

-Reagents: Presence of particulate material, turbidity and absorbance of the blank lower the limit indicated in "Assay parameters".

3. 5. 2. 1. 6 Glucose (Glucose Oxidase/Peroxidase):

Glucose in the sample originates, by means of the coupled reactions described below, a colored complex that can be measured by spectrophotometry[1].

$$\text{Glucose} + \frac{1}{2}O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{Gluconate} + H_2O_2$$

$$2H_2O_2 + \text{phenol} + \text{4-Amino-antipyrine} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4H_2O$$

Composition:

A. Reagent 10 x 50 mL. Phosphate 100 mmol/L, phenol 5 mmol/L, glucose oxidase > 10 U/mL, peroxidase > 1 U/mL, 4-aminoantipyrine o.4 mmol/L, pH 7.5.
B. **Storage:**
C. Store at 2-8 ºC.
D. Reagent is stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.
E. Indications of deterioration:
F. -Reagents: Presence of particulate material, turbidity, absorbance of the blank over the limit indicated in "Assay parameters".

3. 5. 2. 1. 7 Calcium – Arsenazo (ArsenazoIII):

Principle of the method:

Calcium in the sample reacts with arsenazo III forming a coloured complex that can be measured by spectrophotometry[1].

Composition:

A. Reagent 10 x 50 ml. Arsenazo III 0.2 mmol/L, imidazole 75 mmol/L.

Storage:

Store at 2-8 ºC.

Reagent is stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

-Reagents: Presence of particulate material, turbidity.

3. 5. 2. 1. 8 Phosphorus (Phosphomolybdate/UV):

Principle of the method:

Inorganic phosphorus in the sample reacts with molybdate in acid medium forming phosphomolybdate complex that can be measured by spectrophotometry[1,2]

Contents and composition:

A. Reagent: 4 x 60 mL. Sulfuric acid 0.36 mol/L, sodium chloride 154 mmol/L.

   DANGER: H314: Causes severe skin burns and protective gloves/protective clothing/eye protection/face protection. P303 +361+P353: IF ON SKIN (O hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.

B. Reagent: 2 x 50 mL.Sulfuric acid 0.36 mol/L, sodium chloride 154 mmol/L.

   DANGER: H314: Causes severe skin burns and eye damage. P280: Wear protective gloves/protective clothing/eye protection/face protection. P303 +361+P353: IF ON SKIN (ON hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.

Storage:

Store at 15-30 ºC.

Reagent are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

-Reagents: Presence of particulate material, turbidity, absorbance of the blank over 0.500 a 340 nm.

3. 5. 2. 1. 9 Cholesterol HDL:

Principle of the method:

The cholesterol from low density lipoproteins (LDL), very low- density lipoproteins (VLDL) and chylomicrons, is broken down by the cholesterol oxidase in an enzymatic accelerated non-color forming reaction. The detergent present in the reagent B, solubilizes cholesterol from high density lipoproteins (HDL) in the sample. The HDL cholesterol is then spectrophotometrically measured by means of the coupled reactions described below[1].

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Chol.esterase}} \text{Cholestero1} + \text{fatty acid}$$

$$\text{Cholesterol} + {}^{1}/_{2}O_2 + H_2O \xrightarrow{\text{Chol.oxidase}} \text{Cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{DSBmT} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + 4\ H_2O$$

Contents and Composition:

A. Reagent. 3 x 20 mL. Goods buffer, cholesterol oxidase < 1 U/mL, peroxidase < 1 U/mL, N, N-bis (4-sulfobutyl)-m-toluidine (DSBmT) 1 mmol/L, accelerator 1 mmol/L.

B. Reagent. 1 x 20 mL. Goods buffer, cholesterol esterase < 1.5 U/mL, 4-aminoantipyrine 1mmol/L, ascorbate oxidase < 3.0 KU/L, detergent.

Storage:

Store at 2-8 ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration: Presence of particulate material, turbidity.

3. 5. 2. 1 Cholesterol LDL:

Principle of the method:

A specific detergent solubilizes the cholesterol from high density lipoproteins (HDL), very low- density lipoprotein (VLDL) and chylomicrons. The cholesterol esters are broken down by cholesterol esterase and cholesterol oxidase in a non-color forming reaction. The second detergent, present in the reagent B, solubilizes cholesterol from low density lipoproteins (LDL) in the sample. The LDL cholesterol is then spectrophotometrically measured by means of the coupled reactions described below[1].

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Chol.esterase}} \text{Cholestero1} + \text{fatty acid}$$

$$\text{Cholesterol} + {}^{1}/_{2}O_2 + H_2O \xrightarrow{\text{Chol.oxidase}} \text{Cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{DSBmTP} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4\ H_2O$$

Contents and Composition:

A. Reagent. 3 x 20 mL. MES buffer > 30 mmol/L, cholesterol esterase < 1.5 U/mL, cholesterol oxidase < 1.5 U/mL, 4-aminoantipyrine 0.5 mmol/L, ascorbate oxidase < 3.0 U/L, peroxidase > 1 U/mL, detergent, pH 6.3.

B. Reagent. 1 x 20 mL. MES buffer > 30 mmol/L, 1mmol/L, detergent, pH 6.3.

Storage:

Store at 2-8 ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration: Presence of particulate material, turbidity.

3. 5. 2. 1. 1 Uric Acid (Uricase/Peroxidase):

Principle of the method:

Uric acid in the sample originates, by means of the coupled reactions described below, a colored complex that can be measured by spectrophotometry[1,2].

$$\text{Uric acid} + O_2 + H_2O \xrightarrow{\text{uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{DCFS} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4H_2O$$

Composition:

A. Reagent. 10 x 50 mL.phosphate 100 mmol/L, detergent 1. g/L, dichloro-

Phenol-sulfonate 4 mmol/L, uricase> 0.12 U/ml, ascorbate oxidase > 5 U/mL, Peroxidase> 1 U/mL, 4-aminoantipyrine 0.5 mmol/L, pH 7.8.

Storage:

Store at 2-8 ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

-Reagents: Presence of particulate material, turbidity, absorbance of the blank over the limit indicated in “Assay parameters”.

3. 5. 2. 1. 12 Albumin (Bromocresol Green):

Principle of the method:

Albumin in the sample reacts with bromocresol green in acid medium forming a colored complex that can be measured by spectrophotometry[1].

Composition:

Reagent. 5 x 50 mL. Acetate buffer 100 mmol/L, bromocresol green 0.27 mmol/L, detergent, pH 4.1.

Storage:

Reagent (A): Store at 2-8 ºC.

Reagent is stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

A- Reagents: Presence of particulate material, turbidity, absorbance of the blank over the limit indicated in "Assay parameters".

3. 5. 2. 1. 13 Creatinine (Alkaline Picrate):

Principle of the method:

Creatinine in the sample reacts with picrate in alkaline medium forming a colored complex. The complex formation rate is measured in a short period to avoid interferences[1,2].

Composition:

A. Reagent. 5 x 50 mL. Sodium hydroxide 0.2 mol/L, detergent.
   Irritant (Xi): R36/38: Irritating to eyes and skin. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S37/39: Wear suitable gloves and eye/face protection.
B. Reagent. 5 x 50 mL. Picric acid 25 mmol/L.

Storage:

Store at 2-8 ºC.

Reagent is stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

-Reagents: Presence of particulate material, turbidity, absorbance of the blank over 0.350 at 500 nm (1 cm cuvette).

3. 5. 2. 1. 14 Triglycerides (Glycerol Phosphate oxidase/peroxidase):

Principle of the method:

Triglycerides in the sample originates, by means of the coupled reactions described below, a colored complex that can be measured by spectrophotometry[1,2].

$$\text{Triglycerides} + H_2O \xrightarrow{\text{lipase}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{glycerol kinase}} \text{Glycerol -3- P} + \text{ADP}$$

$$\text{Glycerol – 3- P} + O_2 \xrightarrow{\text{G-3-P-oxidase}} \text{Dihydroxyacetones-p} + H_2O_2$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{4- Chlorophenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4H_2O$$

Composition:

A. Reagent: 10 x 50 mL. Pipes 45 mmol/L, magnesium chloride 5 mmol/L, 4-chlorophenol 6 mmol/L, lipase > 100 U/mL, glycerol kinase > 1.5 U/mL, glycerol-3-phosphate oxidase > 4 U/mL, peroxidase > 0.8 U/mL, 4-aminoantipyrine 0.75 mmol/L, ATP 0.9 mmol/L, pH 7.0

Storage:

Store at 2-8 ºC.

Reagent is stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

-Reagents: Presence of particulate material, turbidity, absorbance of the blank over, the limit indicated in “Assay parameters”.

3. 5. 2. 2 Meat Chemical Analysis

3-5-2-2–1 Determination of moisture and dry matter:

Principle:

Moisture as removed from the samples by heating at 105 ºC in a force – draught oven for 3 hour or overnight.

Calculation:

$$\% \text{ moisture} = \frac{\textbf{(Wt of original sample + dish)- (dried sample + dish)}}{\textbf{(Wt of original sample "5 gm")}} \times 100$$

Or

% moisture= 100 - % dry matter

3. 5. 2. 2. 2 Determination of Ash and Organic matter

Principle:

The sample is ignited at 500-550 ºC to burn off all organic materials. The inorganic materials which does not volatilize at that temperature is called Ash. The difference between sample and ash gives the organic matter.

Calculation:

$$\% \text{ Ash} = \frac{\textbf{(wt of Ash + dish)-(wt of dish)}}{} \times 100$$

% organic matter = 100 - % Ash

Nitrogen free Extraction (N.F.E)

%N. F. E = (100- (Moist + Ash + Crude fat + crud protein + Crude fiber)

3. 5. 2. 2. 3 Determination of Crude Fat (soxhlet)

Principle:

The sample is extracted with petroleum spirit, the solvent is distilled off and the extract dried and weighed.

Reagent:

Petroleum spirit, boiling point (60 - 80 c°).

Calculation:

$$\%\text{Crude fat} = \frac{(\text{WT. of flask + oil} - \text{WT. of flask})}{\text{WT. of original sample (2.5)}} \times 100$$

3. 5. 2. 2. 4 Determination of total nitrogen (crude protein) in feed

Principle:

Total nitrogen is determined using the kjeldhal method. Organic nitrogen is converted in to ammonium ions by digestion with concentrated sulphuric acid in the presence of a catalyst such as a mixture of copper sulphate with selenium.

As the digestion proceeds, some of sulphuric acid is reduced to sulphur dioxide which in turn reduces the nitrogenous material to ammonia. The ammonia combines with sulphuric acid to form ammonium sulphate. Ammonia is liberated by boiling with sodium hydroxide, steam distilled in to boric acid plus indicator and determined by titration.

Reagent:

Conc. sulphuric acid

Catalyst (Copper sulphat + selenium)

Sodium hydroxide solution 50%

Standard solution of ammonium sulphate

Standard acid 0.01 N -HCL

Boric acid+ bromocresol green/methyl red indicator solution

Calculation:

$$\% CP = \frac{\text{Titrate-Blank}}{\text{Standard-Blank}} \times \frac{75ml}{3ml} \times \frac{1}{0.5} \times \frac{1}{1000} \times 6.25 \times 100$$

3. 6 Methods used for meat quality assessment:

3. 6. 1 Subjective meat quality attributes

3. 6. 1. 1 Panel Taste

Breast, drumstick and thigh cuts were deboned and frozen then defrosted before cooking for sensory evaluation. Aluminum foil was used for trapping the meat, then placed in roast pan and cooked at 180 C^{o}, and approximately 80 C^{o} internal muscle temperature. The cooked meat was cooled at room temperature for about 10 minutes. Well trained panelists (Ten Persons) were requested to evaluate the cooked samples for: Tenderness, Juiciness, Flavor and Color.

They were advised to drink water between samples evaluated to pause between them. Following recommended procedures (Hawrysh *et al.*, 1980). The sensory panel using eight points scale (Appendix 2).

3. 7 Statistical Analysis

The design used in this study was Complete Randomized Design (C R D). Data of performance, carcass yield, meat quality, serum metabolites and enzyme activities were all analyzed by the Analysis Of Variance (one-way) ANOVA, it was compared between the groups, means were separated by Duncan's multiple range test (Obi., 1990). The level of significant difference set up ($P \leq 0.05$).

CHAPTER FOUR

RESULTS

4. 1 Experiment (1):

Response of broiler chicks fed on graded levels of Eucalyptus and Clove mixed essential oils:

The detected chemical composition of Eucalyptus Essential Oil, reported in Table (4), shows that 46 compounds were identified (Appendix 3), and that the component most present by far is D-(+)-Camphor (21.94%) followed by Cis-2-Menthenol (6.4%), Elemol (6.03%), Cedrenol (5.1%), Gurjunene (4.9%), Cis-Lanceol (4.88%), 4-Carvomenthenol (4.82%), 7-Isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-one (4.65%) and ß-Eudesmol (4.4%).Whereas all the others are present in amounts lower than 2%.

Table (4): Chemical properties of Eucalyptus essential oil:

NO	RT(min)	Name	Area Sum%
1	8.78	D-(+)-Camphor	21.94
2	5.81	Cis-2-Menthenol	6.4
3	11.40	Elemol	6.03
4	11.68	Cedrenol	5.1
5	10.50	Gurjunene	4.9
6	10.64	Cis-Lanceol	4.88
7	7.79	4-Carvomenthenol	4.82
8	12.90	7-Isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-one	4.65
9	12.27	ß-Eudesmol	4.4

*source :(Regional Center for Food and Feed, Ministry of Agriculture, Egypt).

**RT=Retention Time (minute).

The performance results of broilers fed on diets containing graded levels of Eucalyptus and Clove mixed essential oils were illustrated in table (5) and figure (1).

The results showed that no significant ($P \geq 0.05$) differences between chicks fed on control diet and group of chicks fed on 200 mg/kg mixed oils, group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils in body weight, although group of chicks fed on 200 mg/kg mixed oils had the heaviest one than groups of chicks fed on 400 mg/kg mixed oils, group of chicks fed on control diet and group of chicks fed on 600 mg/kg mixed oils. Also no significant ($P \geq 0.05$) differences were observed between all tested groups in feed intake. However the group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils consumed more feed than those group of chicks fed on control diet and group of chicks fed on 400 mg/kg mixed oils during the experimental period. No significant ($P \geq 0.05$) differences between all tested groups in body weight gain through-out the experimental period, however the group of chicks fed on 200 mg/kg mixed oils had numerically the highest body weight gain compared with others tested groups during the experimental period. No significant ($P \geq 0.05$) differences between all tested groups in feed conversion ratio were noticed, but group of chicks fed on 200 mg/kg mixed oils recorded the best feed conversion ratio than group of chicks fed on control diet, group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils.

No mortalities were recorded in all treatment groups through-out the experimental period.

Table (5): Effect of adding graded levels of Eucalyptus and Clove mixed essential oils on the performance of the broilers:

Treatments	Initial body weight (g)	Final body weight (g)	Feed intake (g)	Body weight gain (g)	Feed conversion ratio
Control	187	1942	3428	1757	1.95
200mg/kg	189	2095	3494	1910	1.83
400 mg/kg	189	1986	3314	1801	1.84
600 mg/kg	186	1917	3467	1733	2.03
SE± **Sig**	_	157.77 N.S	122.14 N.S	157.60 N.S	0.138 N.S

Values are mean± SD, Means value(s) bearing no different superscript(s) in a column are not significantly different (P≥0.05) according to Duncan's multiple range test (DMRT), SE ± Standard Error

N.S= non-significant

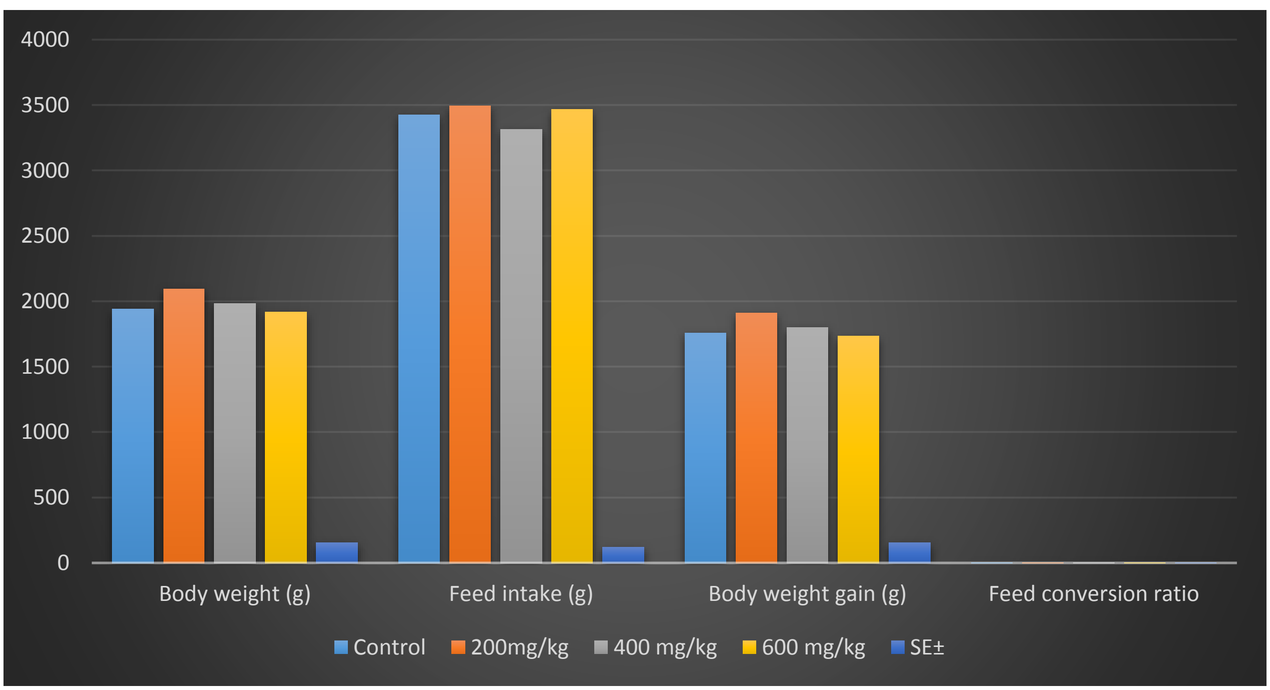


Fig.1: Effect of adding graded levels of Eucalyptus and Clove mixed essential oils on the performance of the broilers

The effect of adding graded levels of Eucalyptus and Clove mixed essential oils on dressing and giblets were illustrated in table (6) and figure (2).

No significant ($P \geq 0.05$) differences for dressing percentage between all tested groups, although group of chicks fed on 200 mg/kg mixed essential oils had the highest value than that in other tested groups, also no significant ($P \geq 0.05$) difference for internal organs (liver, heart and gizzard) between all tested groups observed.

Table (6): Effect of adding graded levels of Eucalyptus and Clove mixed essential oils on dressing and giblets (Liver, Heart and Gizzard):

Treatmens	Dressing%	Liver%	Heart%	Gizzard%
Control	70.310	2.047	0.510	1.547
200 mg/kg	70.433	1.740	0.483	1.573
400 mg/kg	69.767	2.237	0.623	1.703
600 mg/kg	68.467	2.073	0.610	1.300
SE±	2.017	0.210	0.121	0.269
Sig	N.S	N.S	N.S	N.S

Values are mean± SD

Means value(s) bearing no different superscript(s) in a column are not significantly different

(P≥0.05) according to DMRT

SE ± Standard Error N.S = non-significant

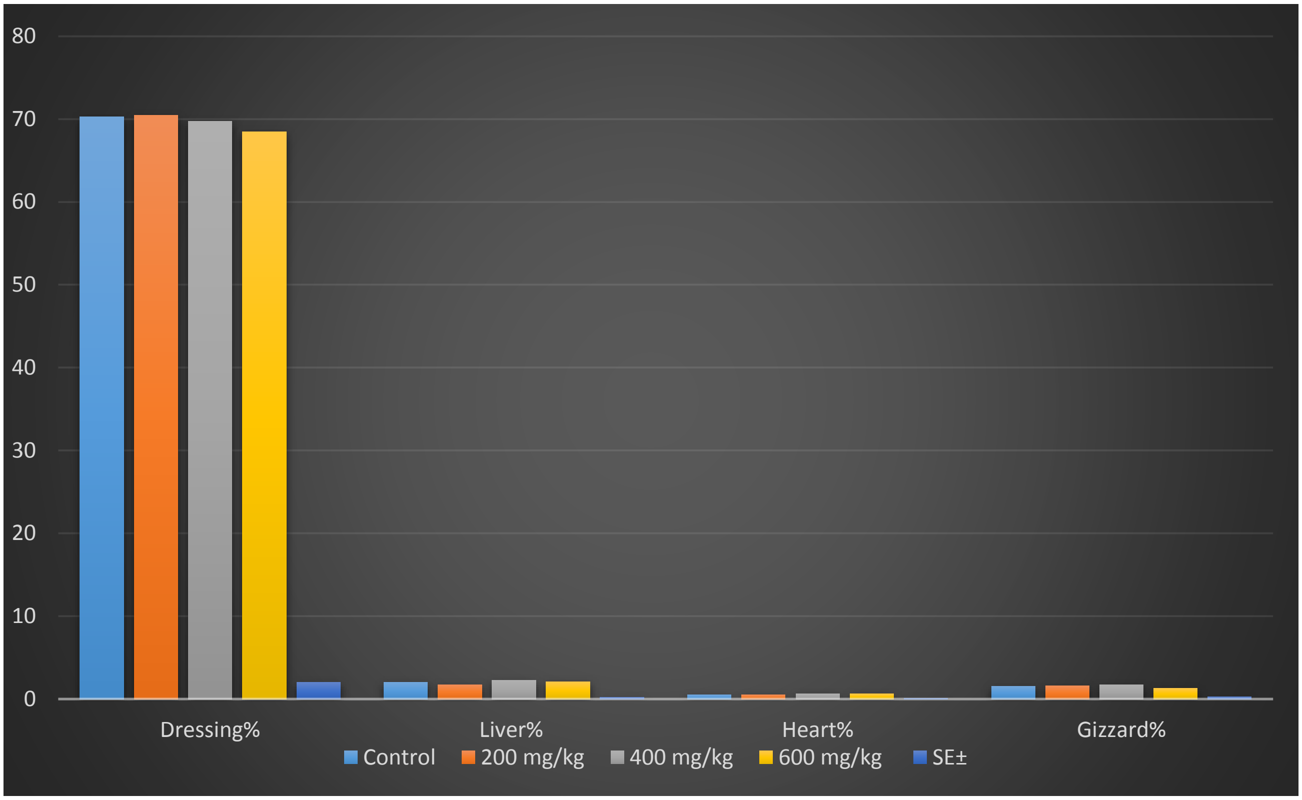


Fig. 2: Effect of adding graded levels of Eucalyptus and Clove mixed essential oils on dressing and giblets (Liver, Heart and Gizzard)

The results of adding graded levels of Eucalyptus and Clove mixed essential oils on non-carcass were showed in table (7) and figure (3).

No significant ($P \geq 0.05$) differences were noticed for head, legs, lung, abdominal fat, intestine weight, back, wing and neck among all tested groups, also no significant ($P \geq 0.05$) differences for kidney between group of chicks fed on control diet, group of chicks fed on 200 mg/kg and 400 mg/kg mixed essential oils compared with group of chicks fed on 600 mg/kg mixed oils, also there was no significant ($P \geq 0.05$) difference for intestine length between group of chicks fed on control diet and 400 mg/kg mixed oils compared with group of chicks fed on 200 mg/kg mixed oils which recorded the shortest and group of chicks fed on 600 mg/kg mixed oils which recorded the longest.

Table (7): Effect of adding graded levels of Eucalyptus and Clove mixed essential oils on non-carcass:

Treatments	Head %	Legs %	Lung %	Kidney %	Abdominal fat%	Intestine weight%	Intestine length%	Back %	Wing %	Neck %
Control	2.553	3.620	0.760	0.373^{b}	1.067	3.800	178.33^{b}	19.693	10.467	5.193
200 mg/kg	2.373	3.497	0.560	0.297^{b}	0.957	3.277	162.67^{c}	19.067	11.967	5.177
400 mg/kg	2.240	3.713	0.757	0.407^{b}	1.170	4.100	171.33^{bc}	22.633	10.167	4.910
600 mg/kg	2.363	3.553	0.733	0.747^{a}	0.780	3.007	200.00^{a}	20.167	10.800	4.740
SE±	0.209	0.239	0.085	0.054	0.286	0.468	4.509	0.988	0.609	0.372
Sig	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

Values are mean± SD

Means value(s) bearing no different superscript(s) in a column are not significantly different (P≥0.05) according to DMRT

SE ± Standard Error N.S = non-significant

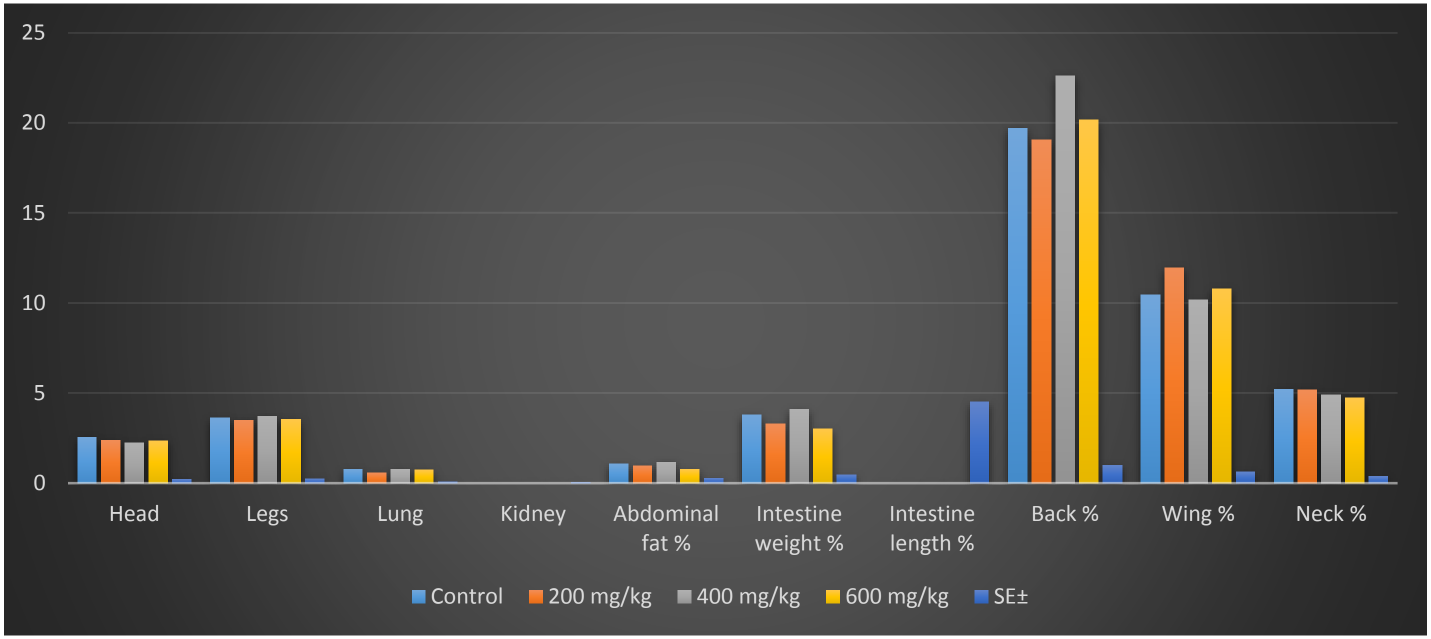


Fig.3: Effect of adding graded levels of Eucalyptus and Clove mixed essential oils on non-carcass

The results of feeding graded levels of Eucalyptus and Clove mixed essential oils on commercial cuts were showed in table (8) and figure (4), their meat values were showed in table (9) and figure (5).

No significant ($P \geq 0.05$) differences between all tested groups in commercial cuts were showed, however group of chicks fed on 400 mg/kg mixed oils had the lowest percentage in drumstick and thigh commercial cuts compared with others tested groups.

Also no significant ($P \geq 0.05$) differences were observed between all tested groups in commercial cuts meat, however group of chicks fed on 200 mg/kg mixed oils had the highest value in breast and drumstick meat compared with other tested groups.

Table (8): Effect of adding graded levels of Eucalyptus and Clove mixed essential oils on commercial cuts:

Treatments	Breast%	Drumstick%	Thigh%
Control	39.460	11.730	15.067
200 mg/kg	38.740	12.333	14.017
400 mg/kg	37.887	11.713	13.233
600 mg/kg	36.087	12.053	14.077
SE±	1.338	1.031	0.777
Sig	N.S	N.S	N.S

Values are mean± SD, Means value(s) bearing no different superscript(s) in a column Are not significantly different (P≥0.05) according to DMRT, SE ± Standard Error

N.S = non-significant

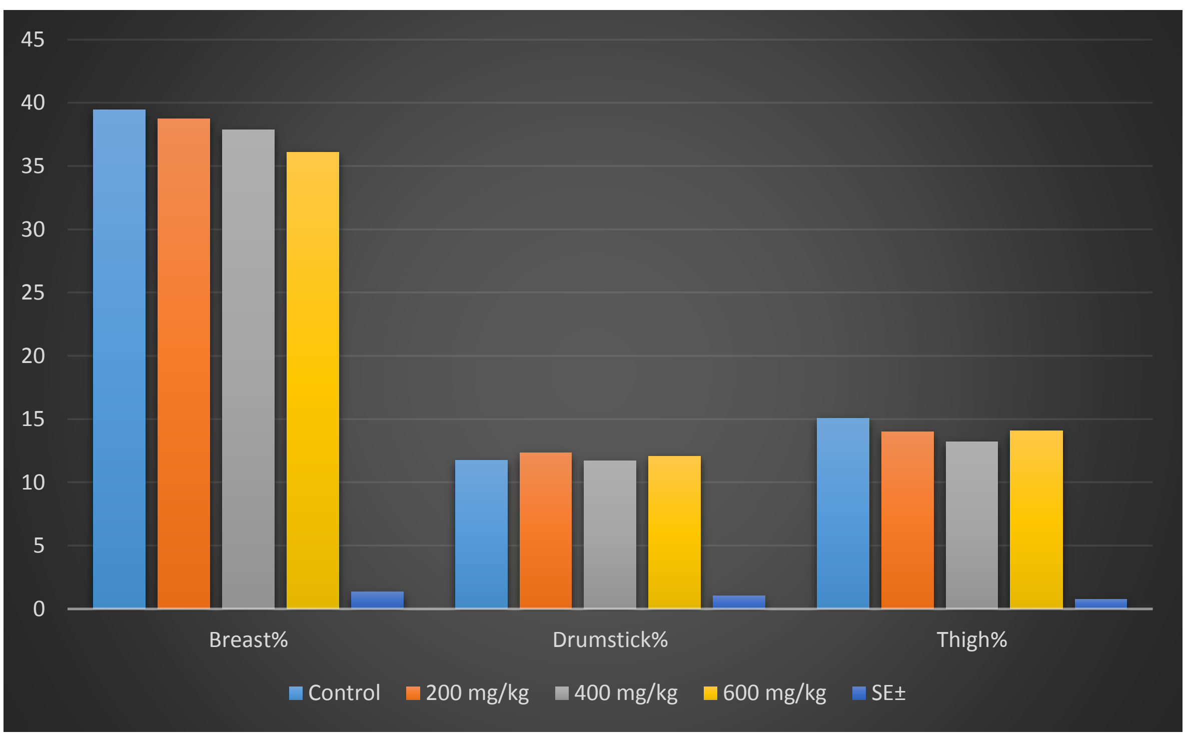


Fig.4: Effect of adding graded levels of Eucalyptus and Clove mixed essential oils on commercial cuts

Table (9): Effect of adding graded levels of Eucalyptus and Clove mixed essential oils on commercial cuts meat:

Treatments	Breast meat%	Drumstick meat %	Thigh meat%
Control	85.150	72.533	84.783
200 mg/kg	87.100	73.433	82.900
400 mg/kg	85.667	66.867	82.300
600 mg/kg	85.033	69.100	81.133
SE±	2.295	3.350	1.858
Sig	N.S	N.S	N.S

Values are mean± SD Means value(s) bearing no different superscript(s) in a column are not significantly different (P≥0.05) According to DMRT, SE ± Standard Errors N.S = non-significant

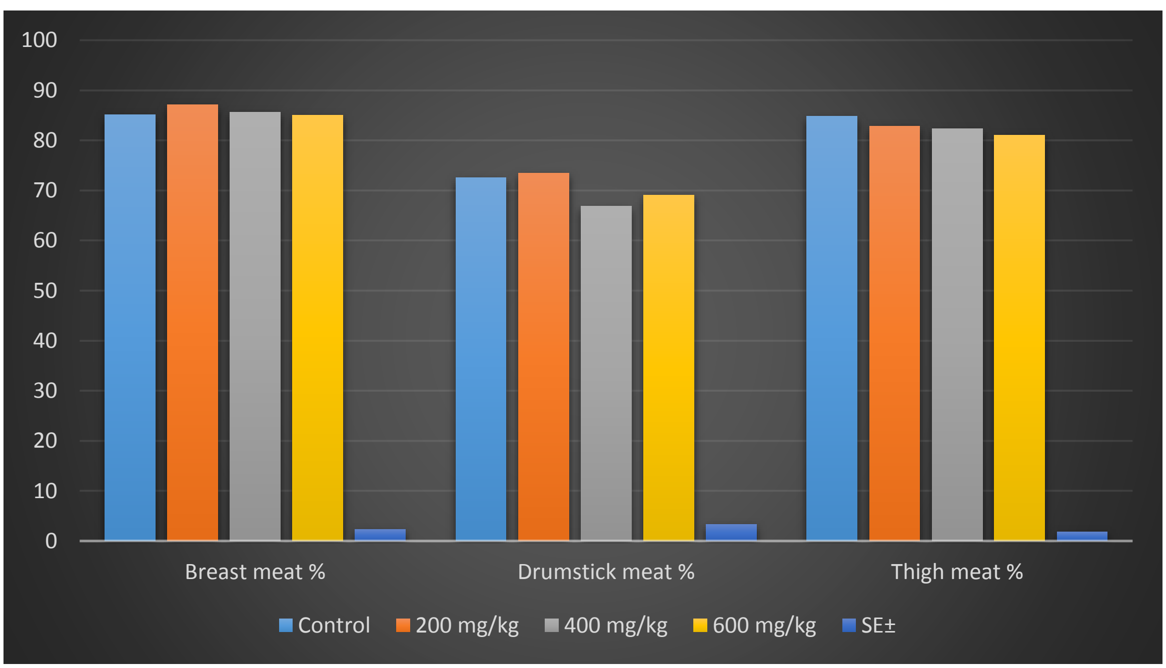


Fig.5: Effect of adding graded levels of Eucalyptus and Clove mixed essential oils on commercial cuts meat

The results of sensory evaluation of chicks' meat fed on graded levels of Eucalyptus and Clove mixed essential oils were illustrated in table (10) and figure (6).

For juiciness no significant (P≥0.05) difference between group of chicks fed on control diet and group fed on 400 mg/kg mixed oils compared with group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils, on the other hand no significant (P≥0.05) differences between group of chicks fed on control diet, group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils in color compared with group of chicks fed on 400 mg/kg mixed oils, also no significant (P≥0.05) difference between group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on control diet in test of flavor, compared with group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils, at the same time there is no significant (P≥0.05) differences between group of chicks fed on control diet, group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils compared with group of chicks fed on 200 mg/kg mixed oils in test of tenderness.

Table (10): Sensory evaluation of chick's meat fed on graded levels of Eucalyptus and Clove mixed essential oils:

Treatments	Juiciness	Color	Flavor	Tenderness
Control	6.130^{a}	6.060^{a}	5.450^{c}	6.110^{ab}
200 mg/kg	5.950^{b}	5.950^{ab}	5.610^{b}	5.640^{c}
400 mg/kg	6.130^{a}	5.690^{c}	5.350^{c}	6.190^{a}
600 mg/kg	5.880^{b}	5.920^{b}	6.060^{a}	5.960^{b}
SE±	0.031	0.037	0.038	0.048

Values are mean± SD

Any two mean value(s) bearing different superscript(s) in a column are significantly different (P≥0.05)

According to DMRT,   SE ± Standard Error

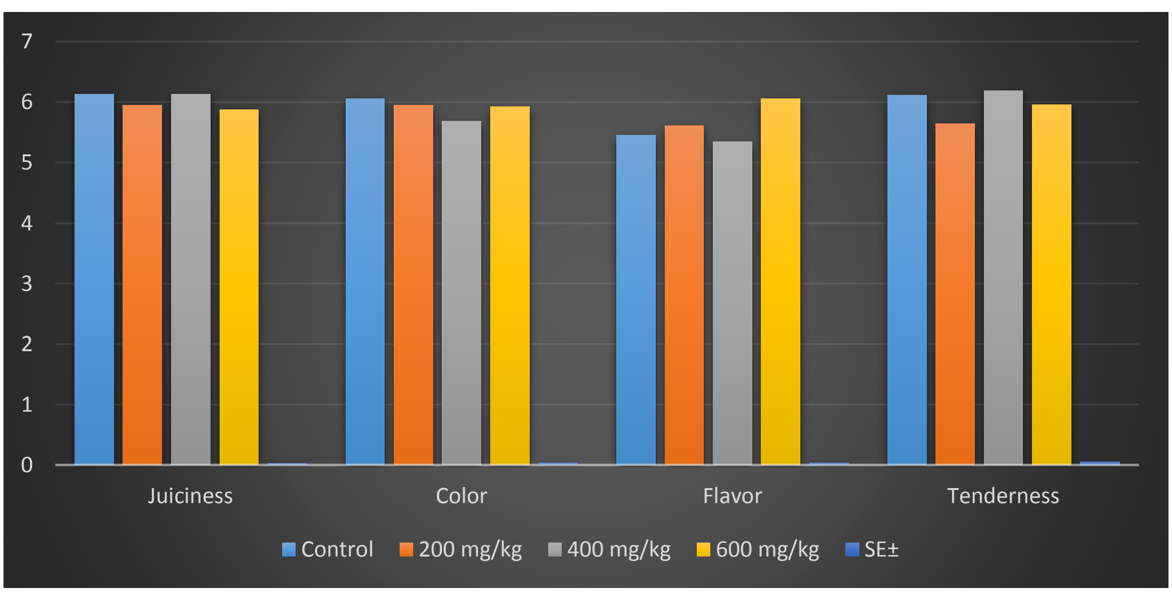


Fig.6: Sensory evaluation of chick's meat fed on graded levels of Eucalyptus and Clove mixed essential oils

The results of adding graded levels of Eucalyptus and Clove mixed essential oils on meat chemical composition were showed in table (11) and figure (7).

No significant ($P \geq 0.05$) difference were observed in moisture between group of chicks fed on control diet, group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 400 mg/kg mixed oils compared with group of chicks fed on 600 mg/kg mixed oils which had the highest. For dry matter no significant ($P \geq 0.05$) difference between group of chicks fed on control diet and group of chicks fed on 200 mg/kg mixed oils, and no significant ($P \geq 0.05$) difference between group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils, no significant ($P \geq 0.05$) differences among all tested groups for ash, also for crude protein there were no significant ($P \geq 0.05$) difference between group of chicks fed on control diet and group of chicks fed on 600 mg/kg mixed oils compared with group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 400 mg/kg mixed oils which had the lowest level, also for ether extract no significant ($P \geq 0.05$) difference between group of chicks fed on control diet and group of chicks fed on 200 mg/kg mixed oils compared with group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils.

Table (11): Effect of adding graded levels of Eucalyptus and Clove mixed essential oils on meat chemical composition:

Treatments	Moisture %	Dry-matter%	Ash %	Crude protein%	Ether extract%
Control	73.233[b]	26.767[a]	1.2000	22.583[a]	1.377[a]
200 mg/kg	73.617[b]	26.383[ab]	1.3000	21.050[b]	1.150[a]
400 mg/kg	74.600[ab]	24.467[c]	1.2500	20.510[c]	0.300[b]
600 mg/kg	75.567[a]	24.700[bc]	1.3500	22.903[a]	0.103[b]
SE±	0.598	0.533	0.079	0.108	0.098

Values are mean± SD

Means value(s) bearing no different superscript(s) in a column are not significantly different (P≥0.05) according to DMRT

SE ± Standard Error

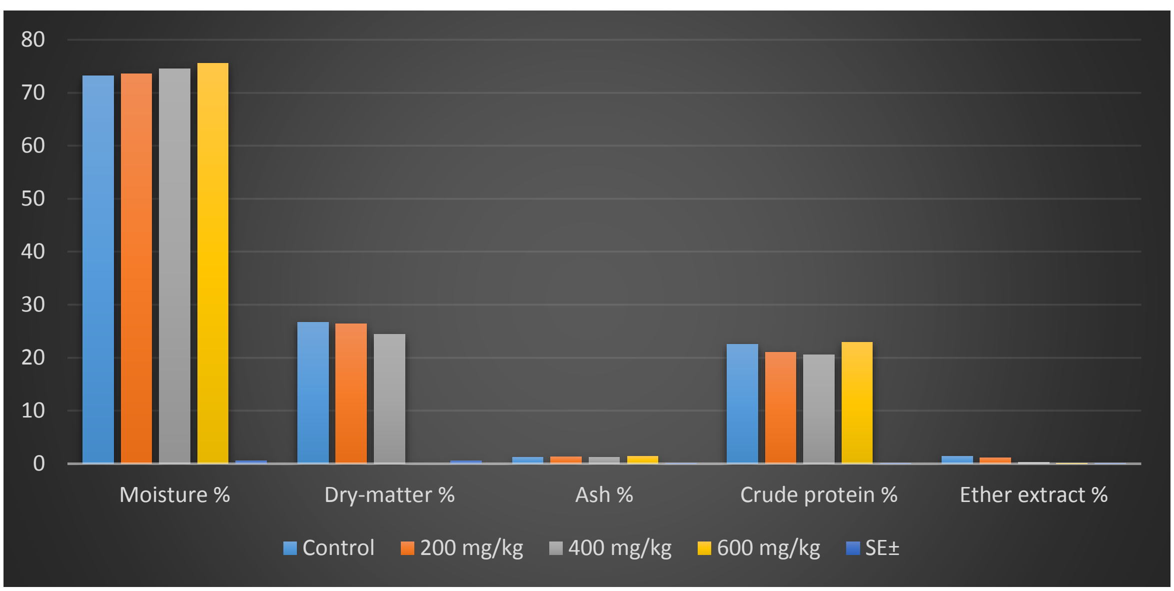


Fig.7: Effect of adding graded levels of Eucalyptus and Clove mixed essential oils on meat chemical composition

The results of adding graded levels of Eucalyptus and Clove mixed essential oils on blood serum metabolites were illustrated in table (12) and figure (8).

For glucose no significant ($P \geq 0.05$) differences between group of chicks fed on 200 mg/kg mixed oils, group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils compared with group of chicks fed on control diet which had the highest level, and for triglyceride no significant ($P \geq 0.05$) difference between group of chicks fed on control diet and group of chicks fed on 200 mg/kg mixed oils compared with group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils which had the lowest, while group of chicks fed on control diet had the highest, also for total protein no significant ($P \geq 0.05$) differences between group of chicks fed on control diet, group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 400 mg/kg mixed oils compared with group of chicks fed on 600 mg/kg mixed oils which had the lowest level, for albumin, no significant ($P \geq 0.05$) differences between group of chicks fed on control diet, group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 400 mg/kg mixed oils compared with group of chicks fed on 600 mg/kg mixed oils which had the lowest, also for cholesterol no significant ($P \geq 0.05$) differences between group of chicks fed on 200 mg/kg mixed oils, group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils compared with group of chicks fed on control diet which had the lowest level, and for cholesterol HDL no significant ($P \geq 0.05$) differences between group of chicks fed on 200 mg/kg mixed oils, group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils compared with group of chicks fed on control diet which had the highest, for cholesterol LDL no significant ($P \geq 0.05$) difference between group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils compared with group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on control diet which had the lowest level, for urea no significant ($P \geq 0.05$) differences were observed between group of chicks fed on 200 mg/kg mixed oils, group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils compared with group of chicks fed on control diet which had the highest, but for uric acid there were significant ($P \leq 0.05$) differences among all tested groups, group of chicks fed on 200 mg/kg mixed oils had the highest level and group of chicks fed on control diet had the lowest, compared with group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils, and for creatinine no significant ($P \leq 0.05$) differences among all tested groups.

Table (12): Effect of adding graded levels of Eucalyptus and Clove mixed essential oils on blood serum metabolites:

Treatments	Glucose (mg\dl)	Tri-gly Ceride (mg\dl)	Protein (g/dl)	Alb. (g/dl)	Cholest. (mg/dl)	Chol. HDL (mg/dl)	Chol. LDL (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
Control	220.50^{a}	43.500^{a}	3.950^{a}	2.050^{bc}	124.50^{c}	130.50^{a}	22.500^{c}	7.100^{a}	3.450^{d}	2.100
200 mg/kg	215.00^{b}	41.000^{a}	3.400^{ab}	2.400^{a}	144.00^{b}	103.50^{b}	39.000^{a}	5.500^{bc}	8.800^{a}	2.100
400 mg/kg	213.50^{b}	34.500^{b}	3.500^{ab}	2.200^{ab}	146.50^{ab}	103.00^{b}	31.000^{b}	5.000^{c}	6.600^{b}	2.100
600 mg/kg	214.00^{b}	32.100^{b}	3.050^{b}	1.950^{c}	149.50^{a}	105.00^{b}	41.500^{a}	6.000^{b}	5.800^{c}	2.030
SE±	0.957	0.848	0.194	0.065	1.061	1.708	0.866	0.208	0.061	0.071

Values are mean± SD

Any two mean value(s) bearing different superscript(s) in a column are significantly different (P≥0.05) according to DMRT

SE ± Standard Error

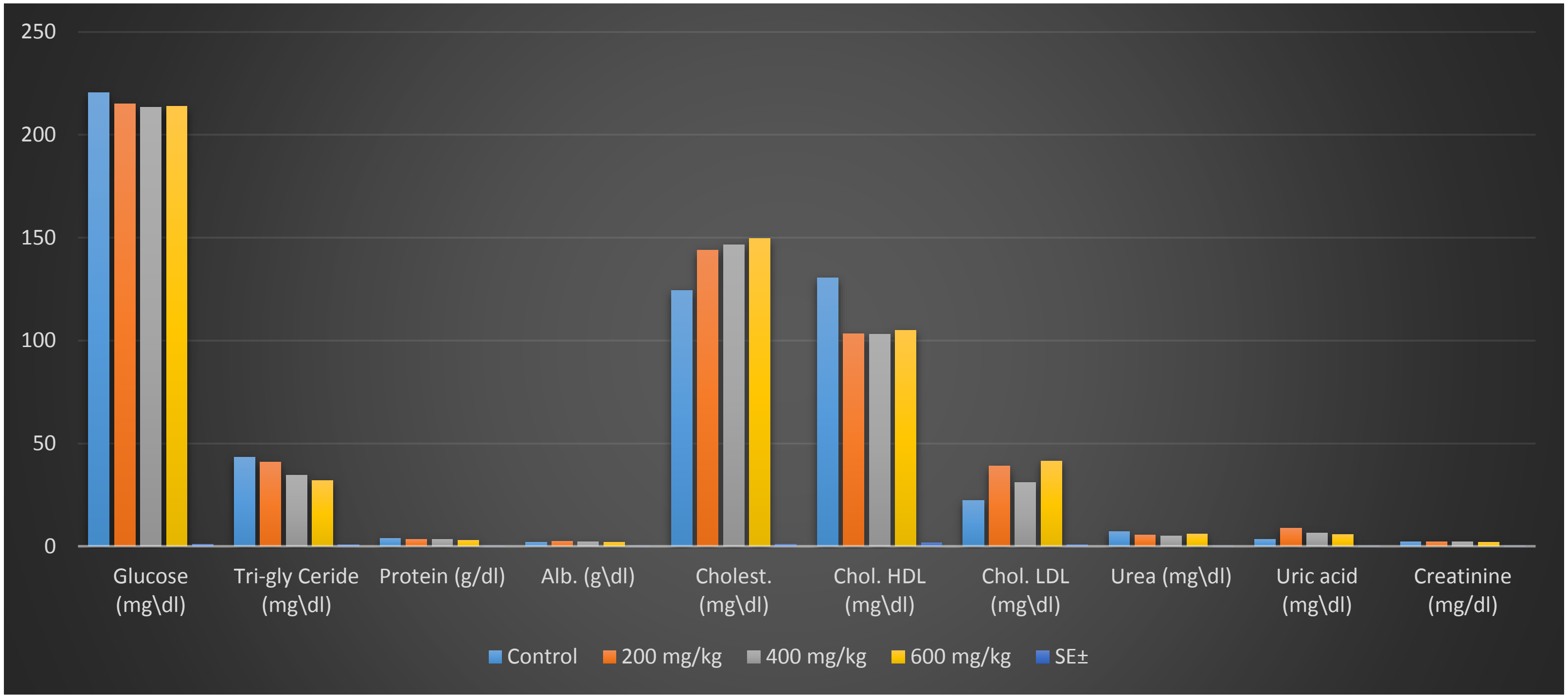


Fig.8: Effect of adding graded levels of Eucalyptus and Clove mixed essential oils on blood serum metabolites

The results of adding graded levels of Eucalyptus and Clove mixed essential oils on serum enzymes and minerals were shown in table (13) and figure (9).

No significant ($P \geq 0.05$) differences for Aspartate Amino Transferase (AST) enzyme between group of chicks fed on control diet, and group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils compared with group of chicks fed on 200 mg/kg mixed oils which recorded the lowest value. Also for Alkaline Phosphatase (ALP) enzyme no significant ($P \geq 0.05$) difference recorded between group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils compared with group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on control diet which had higher level than others.

There were significant ($P \leq 0.05$) differences for Ca ratio between all tested groups, that group of chicks fed on 200 mg/kg mixed oils had the highest ratio and group of chicks fed on 400 mg/kg mixed oils had the lowest compared with group of chicks fed on control diet and group of chicks fed on 600 mg/kg mixed oils.

There were no significant ($P \geq 0.05$) difference for P ratio between group of chicks fed on control diet and group of chicks fed on 200 mg/kg mixed oils compared with group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils which had the lowest ratio.

Table (13): Effect of adding graded levels of Eucalyptus and Clove mixed essential oils on serum enzymes and minerals:

Treatments	AST (iu/l)	ALP (iu/l)	Ca (mg/dl)	Po (mg/dl)
Control	28.950a	247.50a	8.150c	8.750a
200 mg/kg	26.350b	170.85c	11.950a	8.800a
400 mg/kg	28.450a	190.85b	6.100d	6.500b
600 mg/kg	29.350a	171.55c	8.750b	5.900c
SE±	0.453	1.144	0.106	0.061

Values are mean± SD, Means value(s) bearing no different superscript(s) in a column are not Significantly different (P≥0.05) according to DMRT, SE ± Standard Error

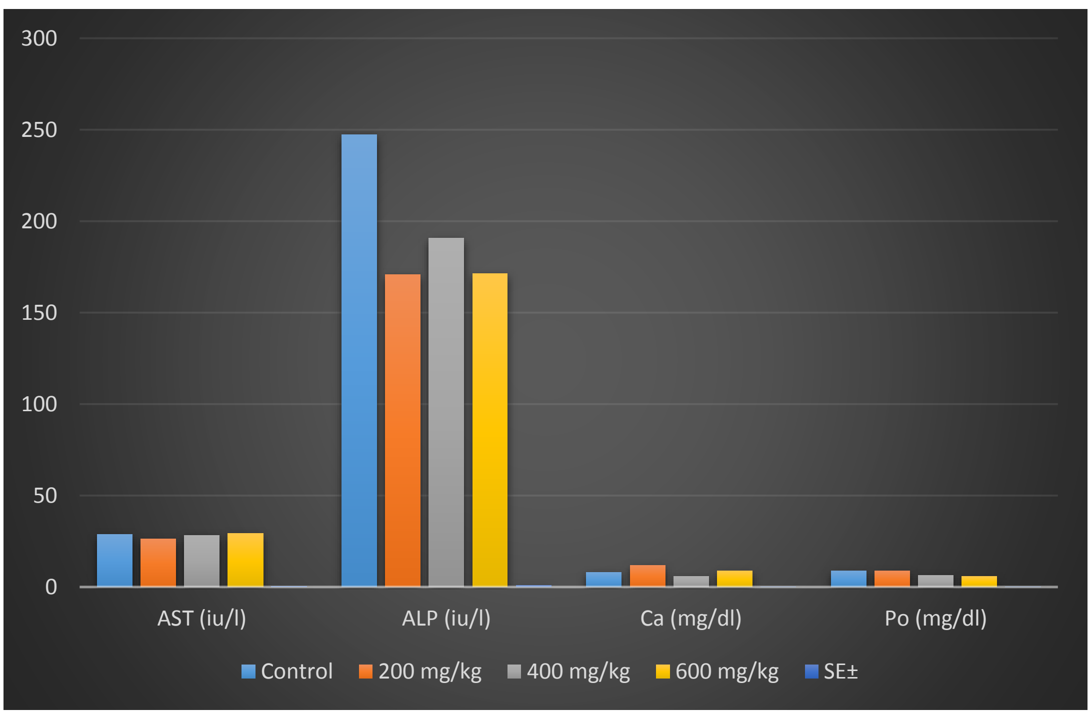


Fig.9: Effect of adding graded levels of Eucalyptus and Clove mixed essential oils on serum enzymes and minerals

Economical Appraisal:

Chicks purchase, feed cost, management, labor and electricity values were the main inputs considered. The total revenues obtained resemble the total selling values of the meat. Profitability ratio (1.149) of group of birds fed on 200 mg/kg mixed oils was the highest ratio compared with other tested groups.

Table (14): Economical appraisal for broiler chicks fed on graded levels of Eucalyptus and Clove mixed essential oils:

Parameters	Control	200 mg/kg	400 mg/kg	600 mg/kg
Chick price	19	19	19	19
Diet cost	37.7	42.0	43.5	48.6
Labor	7	7	7	7
Total cost	63.7	68.0	69.5	74.6
Revenues	137.8	153.2	139.2	140.3
Profit	74.1	85.2	69.7	65.7
Profitability ratio	1	1.149	0.941	0.887

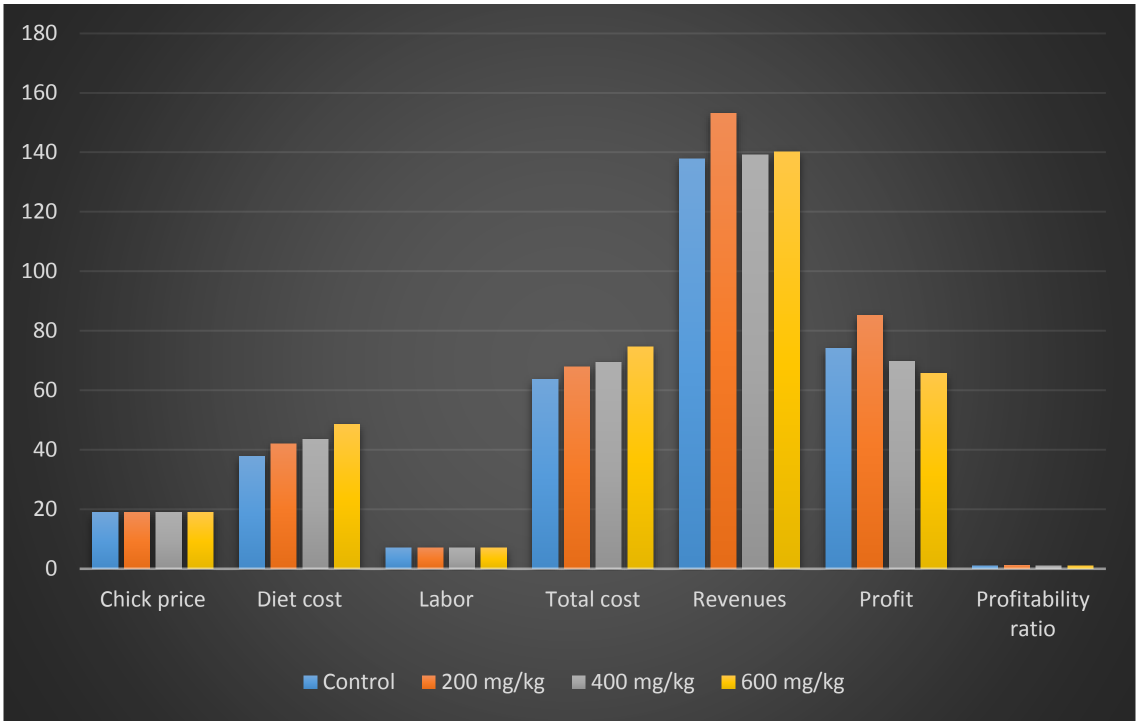


Fig.10: Economical appraisal for broiler chicks fed on graded levels of Eucalyptus and Clove mixed essential oils

4. 2 Experiment (2):

Response of broiler chicks fed on graded levels of Eucalyptus and Basil mixed essential oils:

The detected chemical composition of Basil Essential Oil, reported in Table (15), shows that 43 compounds were identified (Appendix 4), and that the component most present by far is Eugenol methyl ether (21.49%) followed by Linalool (15.66%), Methylisoeugenol (11.96%), Eucalyptol (8.4%), Anethole (4.53%), a-Guaiene (3.98 %), Aromandendrene (2.94%), Y-Gurjunene (2.91%), Chavicol methyl ether (2.47%), Isoledene (2.25%), ß-Pinene (2.17%).Whereas all the others are present in amounts lower than 2%.

Table (15): Chemical properties of Basil essential oil:

NO	RT(min)	Name	Area Sum%
1	10.09	Eugenol methyl ether	21.49
2	7.32	Linalool	15.66
3	10.44	Methylisoeugenol	11.96
4	6.09	Eucalyptol	8.4
5	8.25	Anethole	4.53
6	11.14	a-Guaiene	3.98
7	10.64	Aromandendrene	2.94
8	12.26	Y-Gurjunene	2.91
9	7.97	Chavicol methyl ether	2.47
10	11.27	Isoledene	2.25
11	5.28	ß-Pinene	2.17

*source :(Regional Center for Food and Feed, Ministry of Agriculture, Egypt).

**RT=Retention Time (minute).

The performance results for broiler chicks fed on diets containing graded levels of Eucalyptus and Basil mixed essential oils were illustrated in table (16) and figure (11).

For body weight no significant (P≥0.05) differences among all tested groups, however group of chicks fed on 600 mg/kg mixed oils had the highest weight and numerically group of chicks fed on control diet had the lowest value, compared with group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 400 mg/kg mixed oils. For feed intake no significant (P≥0.05) differences were observed among all tested groups, however group of chicks fed on 600 mg/kg mixed oils had the highest, compared with group of chicks fed on 200 mg/kg mixed oils, group of chicks fed on control diet and group of chicks fed on 400 mg/kg mixed oils which had the lowest. For body weight gain no significant (P≥0.05) differences observed among all tested groups, however group of chicks fed on 200 mg/kg mixed oils had the highest weight gain and group of chicks fed on control diet had the lowest, compared with group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils.

No significant (P≥0.05) differences showed for feed conversion ratio between all tested groups, however group of chicks fed on 200 mg/kg mixed oils had the best feed conversion ratio compared with group of chicks fed on 400 mg/kg mixed oils, group of chicks fed on 600 mg/kg mixed oils and group of chicks fed on control diet.

Table (16): Effect of adding graded levels of Eucalyptus and Basil mixed essential oils on the performance of the broilers:

Treatments	Initial body weight (g)	Final body weight (g)	Feed intake (g)	Body weight gain (g)	Feed conversion ratio
Control	187	1942.o	3428.7	1757.0	1.9500
200 mg/kg	184	2211.0	3478.3	2026.0	1.7233
400 mg/kg	184	2065.7	3269.7	1880.7	1.7433
600 mg/kg	187	2441.0	3517.0	1922.7	1.8300
SE± **Sig**	_	203.70 N.S	201.67 N.S	109.01 N.S	0.1103 N.S

Values are mean± SD, Means value(s) bearing no different superscript(s) in a column are not significantly different (P≥0.05) according to DMRT, SE ± Standard Error N.S = non-significant

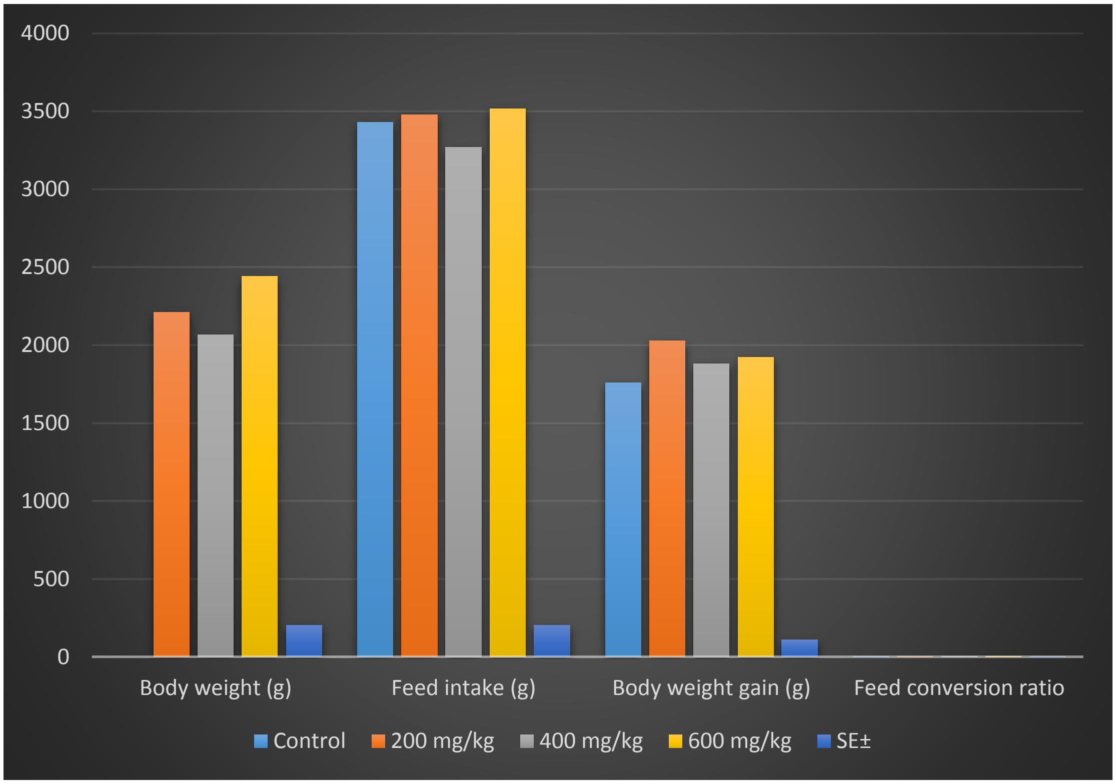


Fig.11: Effect of adding graded levels of Eucalyptus and Basil mixed essential oils on the performance of the broiler chicks

The effect of adding graded levels of Eucalyptus and Basil mixed essential oils on dressing and giblets were illustrated in table (17) and figure (12).

No significant ($P \geq 0.05$) differences were observed between all tested groups for dressing percentage, however group of chicks fed on 600 mg/kg mixed oils had the highest percentage compared with other groups, and group of chicks fed on control diet had the lowest value. On the other hands no significant ($P \geq 0.05$) differences for liver and heart percentage between all tested groups, but for gizzard no significant ($P \geq 0.05$) differences between group of chicks fed on control diet, group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 400 mg/kg mixed oils compared with group of chicks fed on 600 mg/kg mixed oils which showed the lowest value.

Table (17): Effect of adding graded levels of Eucalyptus and Basil mixed essential oils on dressing and giblets (Liver, Heart and gizzards):

Treatments	Dressing%	Liver%	Heart%	Gizzard%
Control	70.310	2.047	0.510	1.547^{ab}
200 mg/kg	69.183	1.943	0.520	1.327^{ab}
400 mg/kg	67.587	2.323	0.520	1.683^{a}
600 mg/kg	71.050	1.960	0.577	1.280^{b}
SE± **Sig**	1.564 N.S	0.242 N.S	0.059 N.S	0.121

Values are mean± SD, Means value(s) bearing no different superscript(s) in a column are not significantly different (P≥0.05) according to DMRT, SE ± Standard Error N.S = non-significant

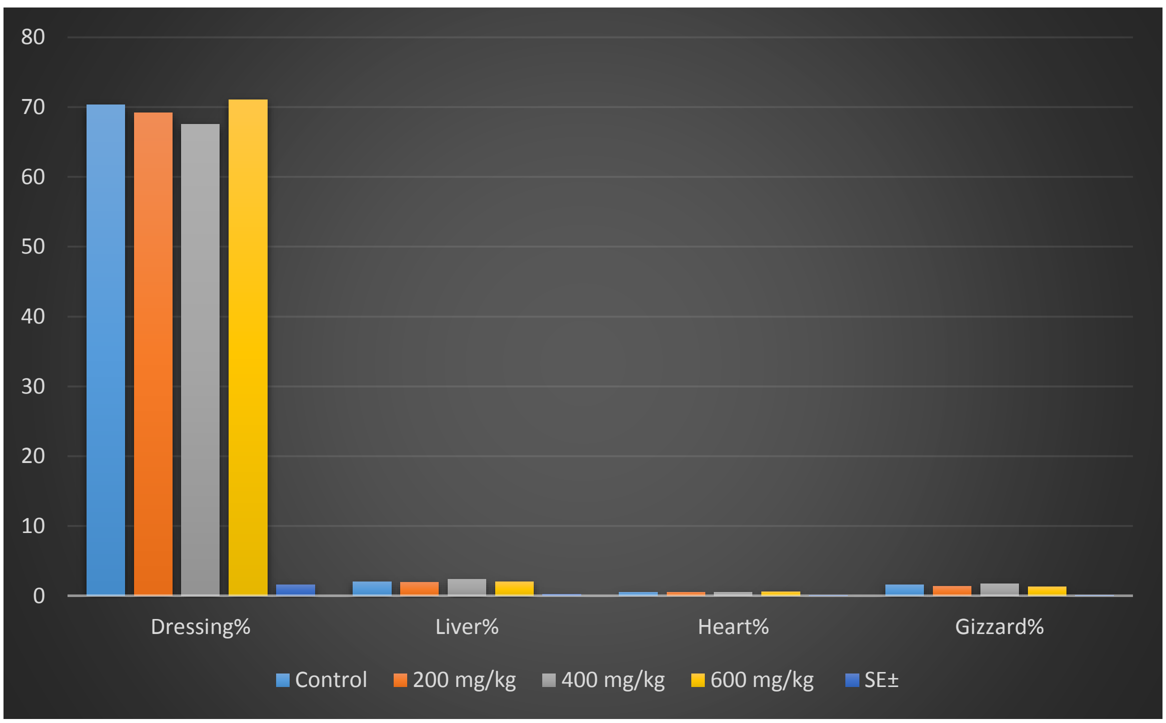


Fig.12: Effect of adding graded levels of Eucalyptus and Basil mixed essential oils on dressing and giblets (Liver, Heart and gizzards)

The effect of adding graded levels of Eucalyptus and Basil mixed essential oils on non-carcass were showed in table (18) and figure (13).

There were no significant ($P \geq 0.05$) differences observed between all tested groups in head, legs, lung, abdominal fat, intestine weight and length, wing and neck, but for kidney, group of chicks fed on 600 mg/kg mixed oils recorded positive significant ($P \leq 0.05$) difference compared with other tested groups. For back group fed on 400 mg/kg mixed oils recorded significantly ($P \leq 0.05$) the highest value compared with other tested groups.

Table (18): Effect of adding graded levels of Eucalyptus and Basil mixed essential oils on non-carcass:

Treatments	Head %	Legs %	Lung %	Kidney %	Abdominal fat%	Intestine weight %	Intestine length%	Back %	Wing %	Neck %
Control	2.553	3.620	0.760	0.373^{b}	1.067	3.800	178.33	19.693^{ab}	10.467	5.193
200 mg/kg	2.427	3.880	0.633	0.463^{ab}	0.727	3.700	182.33	20.233^{ab}	9.633	4.617
400 mg/kg	2.203	3.547	0.677	0.467^{ab}	o.947	4.017	182.67	22.267^{a}	10.400	4.930
600 mg/kg	2.193	3.763	0.597	0.543^{a}	1.243	3.727	174.00	18.167^{b}	11.067	5.437
SE± **Sig**	0.125 N.S	0.132 N.S	0.071 N.S	0.043	0.314 N.S	0.516 N.S	4.021 N.S	1.106	0.551 N.S	0.369 N.S

Values are mean± SD

Means value(s) bearing no different superscript(s) in a column are not significantly different (P≥0.05) according to DMRT

SE ± Standard Error N.S = non-significant.

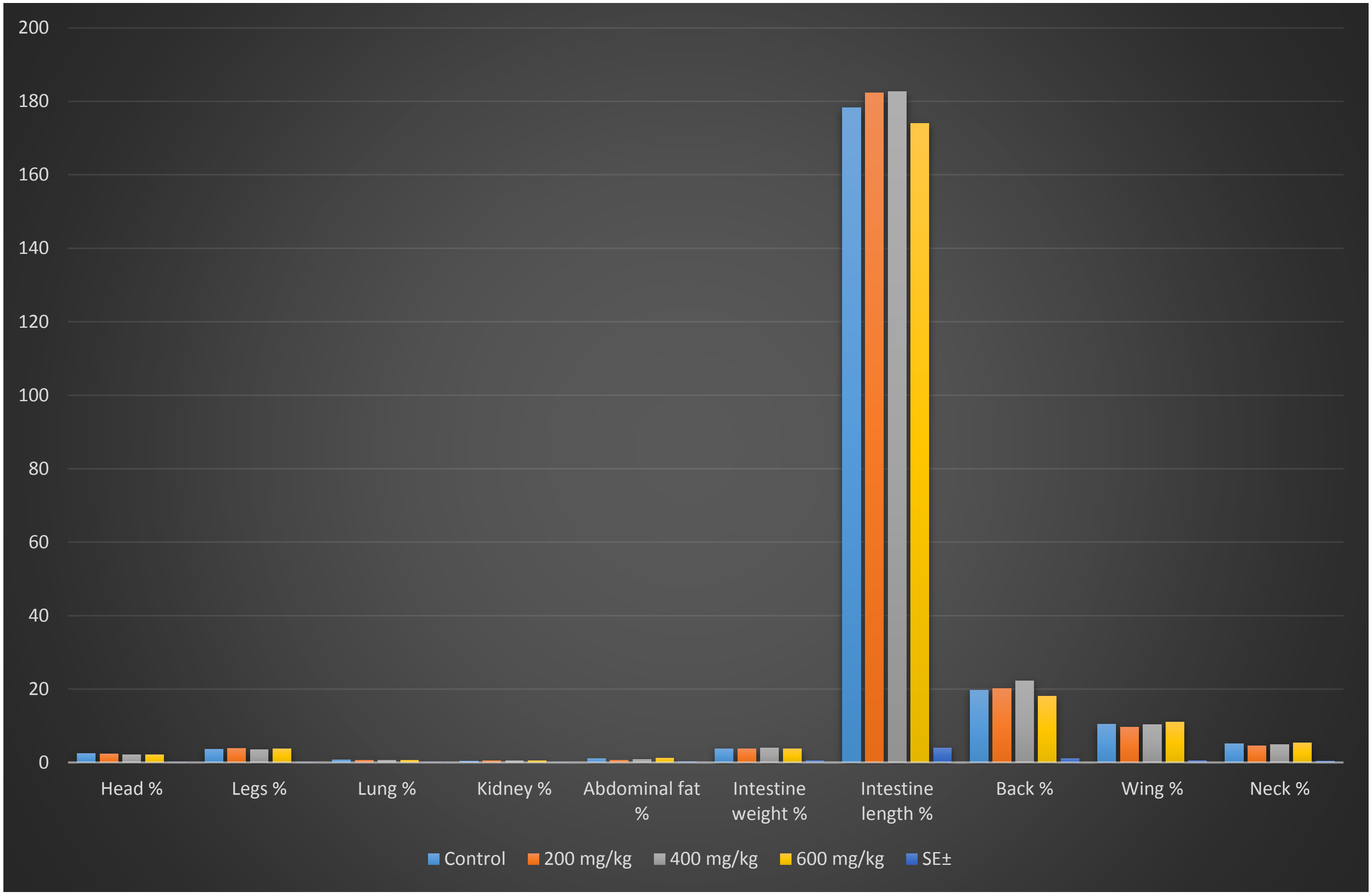


Fig.13: Effect of adding graded levels of Eucalyptus and Basil mixed essential oils on non-carcass

The results of feeding graded levels of Eucalyptus and Basil mixed essential oils on commercial cuts were showed in table (19) and figure (14), their meat values were showed in table (20) and figure (15).

There were no significant ($P \geq 0.05$) differences observed for breast and drumstick percentage between all tested groups, but for thigh no significant ($P \geq 0.05$) difference between group of chicks fed on control diet, group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils compared with group of chicks fed on 400 mg/kg mixed oils which showed significantly ($P \geq 0.05$) the lowest percentage.

No significant ($P \geq 0.05$) differences were observed for meat commercial cuts among all tested groups.

Table (19): Effect of adding graded levels of Eucalyptus And Basil mixed essential oils on commercial cuts:

Treatments	Breast %	Drumstick%	Thigh%
Control	39.460	11.730	15.06^{ab}
200 mg/kg	38.037	13.663	14.74^{ab}
400 mg/kg	36.550	12.807	12.83^{b}
600 mg/kg	38.003	13.090	15.52^{a}
SE±	1.656	0.843	0.729
Sig	N.S	N.S	

Values are mean± SD, Means value(s) bearing no different superscript(s) in a column are not significantly different (P≥0.05) according to DMRT, SE ± Standard Error N.S = non-significant

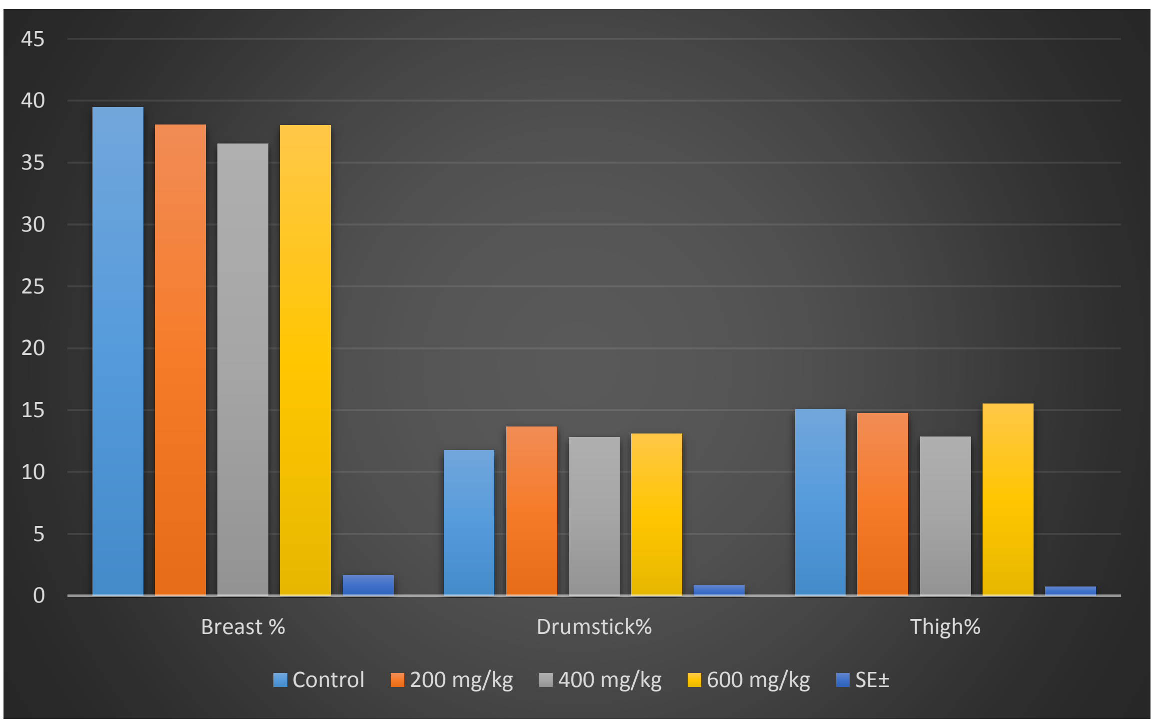


Fig.14: Effect of adding graded levels of Eucalyptus and Basil mixed essential oils on commercial cuts

Table (20): Effect of adding graded levels of Eucalyptus and Basil mixed essential oils on commercial cuts meat:

Treatments	Breast meat%	Drumstick meat%	Thigh meat%
Control	85.150	72.533	84.783
200 mg/kg	86.667	68.567	81.733
400 mg/kg	81.000	72.967	81.500
600 mg/kg	87.400	75.100	82.200
SE±	2.928	3.032	2.211
Sig	N.S	N.S	N.S

Values are mean± SD, Means value(s) bearing no different superscript(s) in a column are not significantly different (P≥0.05) according to DMRT, SE ± Standard Error N.S = non-significant

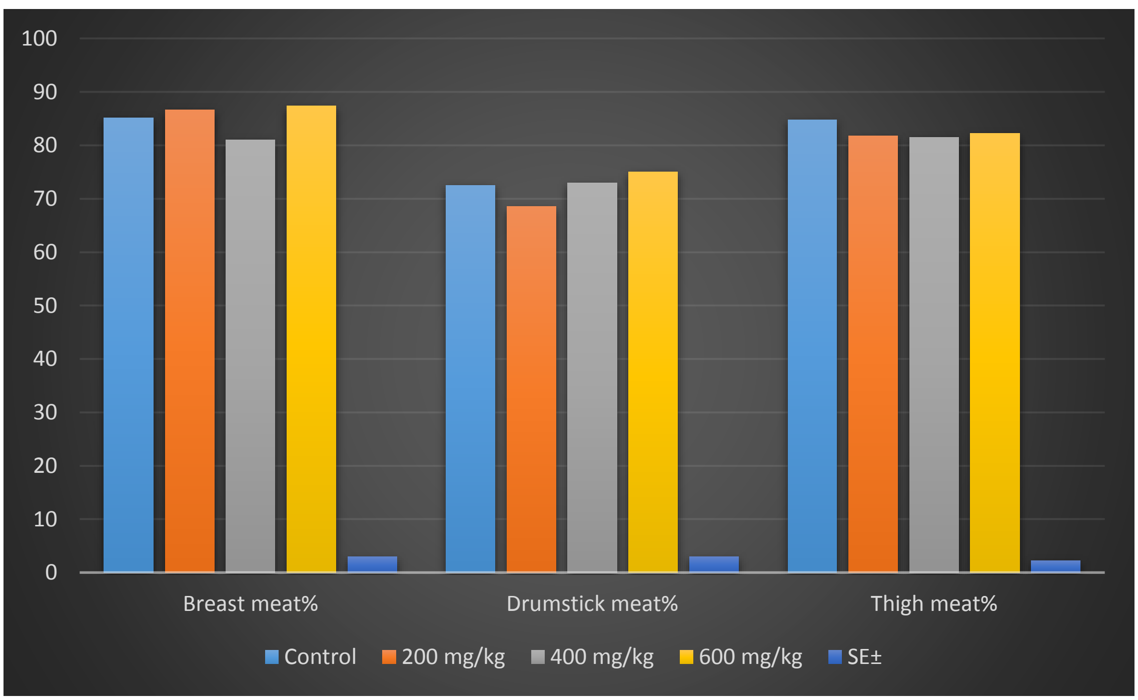


Fig.15: Effect of adding graded levels of Eucalyptus and Basil mixed essential oils on commercial cuts meat

The results of sensory evaluation of chicks' meat fed on graded levels of Eucalyptus and Basil mixed essential oils were illustrated in table (21) and figure (16).

Results obtained for sensory evaluation revealed significant ($P \leq 0.05$) differences for all parameters except tenderness, which showed no significant differences among all tested groups. However group fed on 400 mg/kg mixed oils showed significantly ($P \geq 0.05$) the lowest value for juiciness, which group fed on 600 mg/kg mixed oils showed significantly ($P \geq 0.05$) the best value for color and group fed on 400 mg/kg mixed oils showed significantly ($P \geq 0.05$) the best value for flavor. Values obtained for all parameters were within range.

Table (21): Sensory evaluation of chick's meat fed on graded levels of Eucalyptus and Basil mixed essential oils:

Treatments	Juiciness	Color	Flavor	Tenderness
Control	6.130[a]	6.060[b]	5.450[b]	6.110
200 mg/kg	5.670[ab]	5.590[d]	5.330[b]	5.820
400 mg/kg	5.270[b]	5.780[c]	5.690[a]	5.500
600 mg/kg	5.500[ab]	6.260[a]	5.100[c]	5.990
SE±	0.205	0.031	0.050	0.208

Values are mean± SD

Any two mean value(s) bearing different superscript(s) in a column are significantly different ($P \geq 0.05$)

According to DMRT

SE ± Standard Error

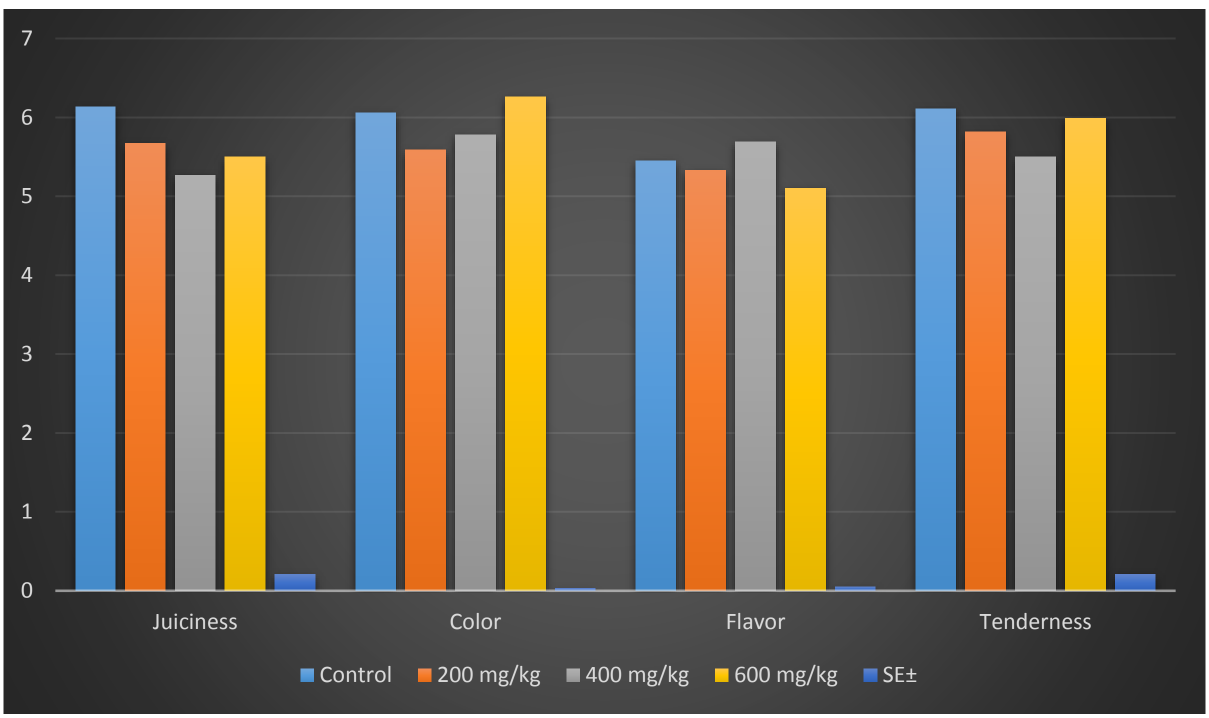


Fig.16: Sensory evaluation of chick's meat fed on graded levels of Eucalyptus and Basil mixed essential oils

The results of adding graded levels of Eucalyptus and Basil mixed essential oils on meat chemical composition were showed in table (22) and figure (17).

For moisture there were no significant (P≥0.05) differences between group of chicks fed on 200 mg/kg mixed oils, group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils compared with group of chicks fed on control diet which had the lowest, and for dry matter no significant (P≥0.05) difference were observed between group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 400 mg/kg mixed oils compared with group of chicks fed on 600 mg/kg mixed oils and group of chicks fed on control diet which had the higher level, also for ash no significant (P≥0.05) difference between group of chicks fed on control diet and group of chicks fed on 400 mg/kg mixed oils, and no significant (P≥0.05) difference between group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils, for crude protein no significant (P≥0.05) difference between group of chicks fed on control diet and group of chicks fed on 400 mg/kg mixed oils compared with group of chicks fed on 200 mg/kg mixed oils which had the highest and group of chicks fed on 600 mg/kg mixed oils which had the lowest, and for ether extract no significant (P≥0.05) difference between group of chicks fed on control diet and group of chicks fed on 200 mg/kg mixed oils compared with group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils which had the lowest.

Table (22): Effect of adding graded levels of Eucalyptus and Basil mixed essential oils on meat chemical composition:

Treatments	Moisture%	Dry-matter%	Ash%	Crude protein%	Ether extract%
Control	73.233^{c}	26.767^{a}	1.200^{b}	22.583^{b}	1.377^{a}
200 mg/kg	75.000^{ab}	25.000^{bc}	1.400^{a}	24.090^{a}	1.150^{a}
400 mg/kg	74.900^{b}	25.100^{b}	1.200^{b}	22.687^{b}	0.600^{b}
600 mg/kg	76.467^{a}	23.533^{c}	1.300^{ab}	20.980^{c}	0.450^{b}
SE±	0.476	0.476	0.061	0.061	0.092

Values are mean± SD, Means value(s) bearing no different superscript(s) in a column are not significantly different (P≥0.05) according to DMRT, SE ± Standard Error

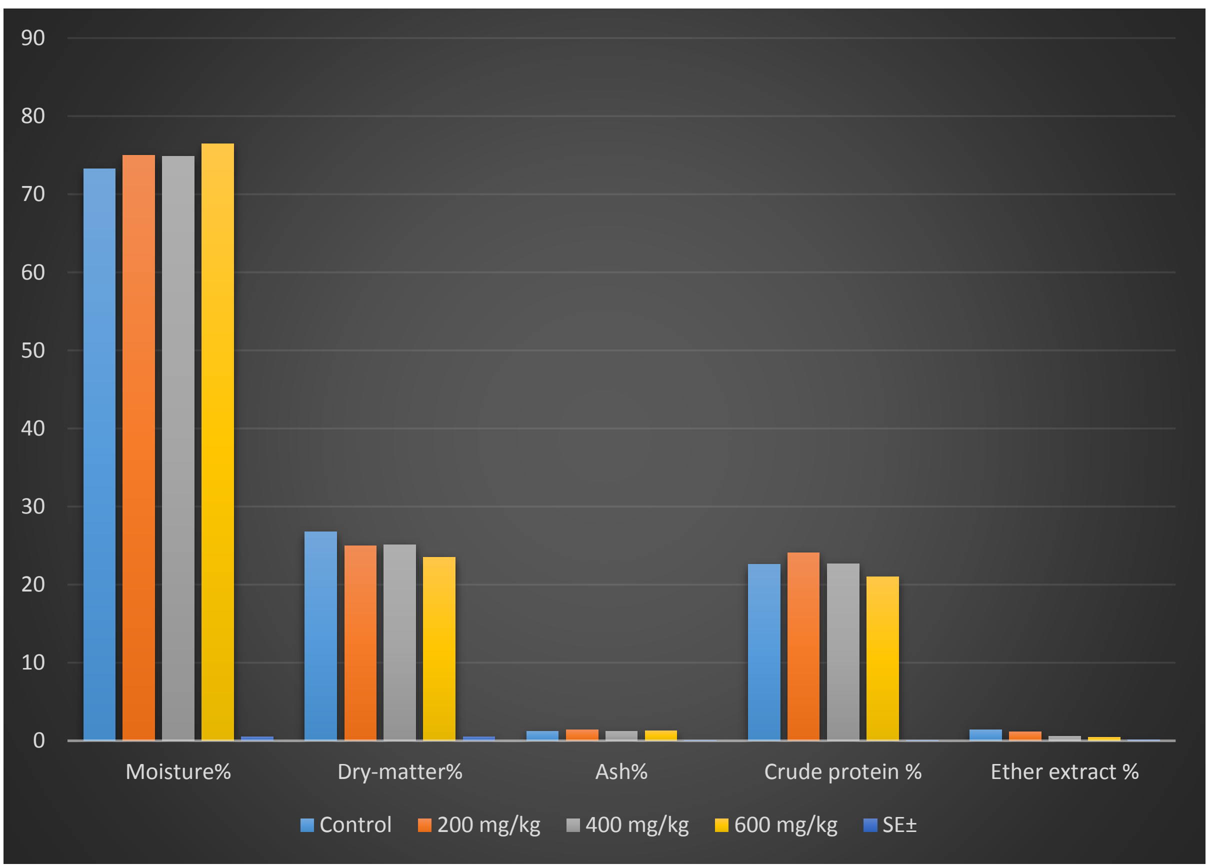


Fig.17: Effect of adding graded levels of Eucalyptus and Basil mixed essential oils on meat chemical composition

The results of adding graded levels of Eucalyptus and Basil mixed essential oils on blood serum metabolites were illustrated in table (23) and figure (18).

For glucose no significant ($P \geq 0.05$) difference between group of chicks fed on control diet and group of chicks fed on 200 mg/kg mixed oils, compared with group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils which had the lowest value, and for triglyceride no significant ($P \geq 0.05$) difference between group of chicks fed on control diet and group of chicks fed on 600 mg/kg mixed oils, compared with group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 200 mg/kg mixed oils which had the lowest level.

For total protein no significant ($P \geq 0.05$) difference between group of chicks fed on control diet which had the highest level and group of chicks fed on 400 mg/kg mixed oils, compared with group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils, also for albumin no significant ($P \geq 0.05$) differences between group of chicks fed on control diet, group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 400 mg/kg mixed oils compared with group of chicks fed on 600 mg/kg mixed oils which had the lowest, for cholesterol no significant ($P \geq 0.05$) differences among all tested groups, but for cholesterol HDL no significant ($P \geq 0.05$) differences between group of chicks fed on 200 mg/kg mixed oils, group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils compared with group of chicks fed on control diet which had the highest, also for LDL no significant ($P \geq 0.05$) difference were observed between group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils, compared with group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on control diet which had the highest. For urea there were significant ($P \leq 0.05$) differences among all tested groups, group of chicks fed on control diet had the highest, while group of chicks fed on 600 mg/kg mixed oils had the lowest, compared with group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 400 mg/kg mixed oils, also for uric acid there were significant ($P \leq 0.05$) differences among all tested groups, group of chicks fed on 400 mg/kg mixed oils had the highest level and group of chicks fed on control diet had the lowest, compared with group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils, On the other hands for creatinine there were no significant ($P \geq 0.05$) difference observed between group of chicks fed on control diet and group of chicks fed on 600 mg/kg mixed oils, compared with group of chicks fed on 200 mg/kg mixed oils which had the lowest level and group of chicks fed on 400 mg/kg mixed oils which had the highest.

Table (23): Effect of adding graded levels of Eucalyptus and Basil mixed essential oils on blood serum metabolites:

Treatments	Glucose (mg\dl)	Tri-gly Ceride (mg\dl)	Protein (g/dl)	Alb. (g/dl)	Cholest (mg/dl)	Chol. HDL (mg/dl)	Chol. LDL (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Creatine (mg/dl)
Control	220.50[a]	43.500[a]	3.950[a]	2.050[ab]	124.50	130.50[a]	22.500[a]	7.100[a]	3.450[d]	2.100[ab]
200 mg/kg	222.00[a]	29.500[b]	2.800[b]	2.200[a]	127.00	127.00[ab]	19.000[b]	4.000[b]	5.950[b]	2.010[b]
400 mg/kg	197.00[b]	30.000[b]	3.800[a]	2.200[a]	125.00	124.50[b]	15.000[c]	3.000[c]	6.750[a]	2.200[a]
600 mg/kg	195.50[b]	40.000[a]	2.800[b]	1.900[b]	126.00	126.00[b]	16.500[c]	2.500[d]	4.550[c]	2.130[ab]
SE±	3.619	1.500	0.061	0.061	1.369	1.323	0.646	0.091	0.134	0.058

Values are mean± SD

Any two mean value(s) bearing different superscript(s) in a column are significantly different (P≥0.05)

According to DMRT

SE ± Standard Error

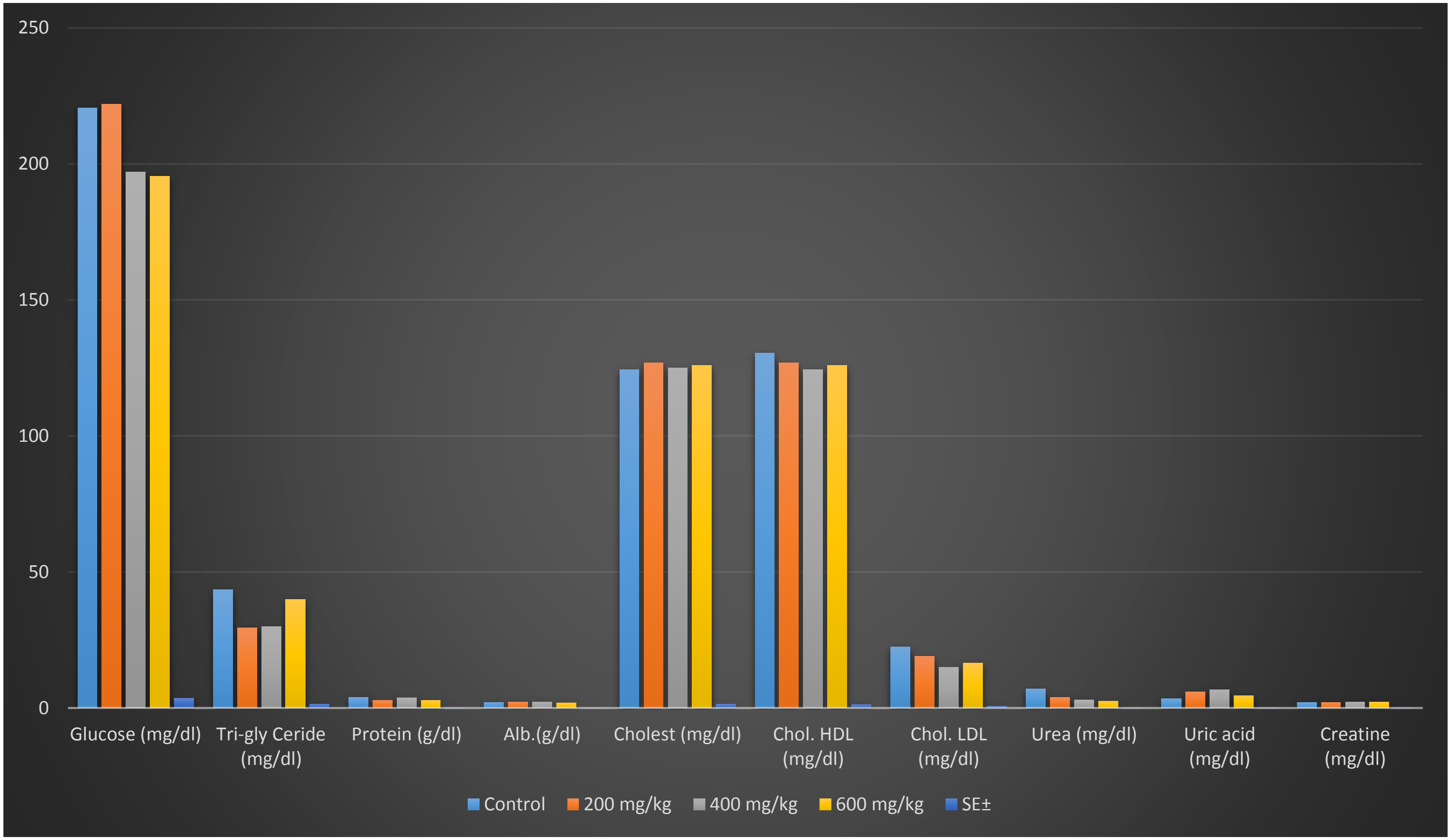


Fig.18: Effect of adding graded levels of Eucalyptus and Basil mixed essential oils on blood serum metabolites

The results of adding graded levels of Eucalyptus and Basil mixed essential oils on blood serum enzymes and minerals were showed in table (24) and figure (19).

For Aspartate Amino Transferase (AST) no significant ($P \geq 0.05$) difference between group of chicks fed on control diet and these fed on 400 mg/kg mixed oils, compared with group fed on 200 mg/kg mixed oils and group fed on 600 mg/kg mixed oils which had the highest level, but for Alkaline Phosphatase (ALP) there were significant ($P \leq 0.05$) differences among all tested groups, group of chicks fed on control diet had the highest level, while group fed on 400 mg/kg mixed oils had the lowest, compared with group fed on 200 mg/kg mixed oils and group fed on 600 mg/kg mixed oils.

For Ca no significant ($P \geq 0.05$) difference between group of chicks fed on control diet and group fed on 600 mg/kg mixed oils, compared with group of chicks fed on 200 mg/kg mixed oils and group fed on 400 mg/kg mixed oils which had the highest value. Results obtained for P showed significant ($P \leq 0.05$) differences among all tested groups, however P level decreased significantly ($P \geq 0.05$) with the increasing mixed oils level in the diet, group of chicks fed on control diet had the highest level, while group fed on 600 mg/kg mixed oils had the lowest compared with other tested groups.

Table (24): Effect of adding graded levels of Eucalyptus and Basil mixed essential oils on blood serum enzymes and minerals:

Treatments	AST (iu/l)	ALP (iu/l)	Ca (mg/dl)	Po (mg/dl)
Control	28.950^{ab}	247.50^{a}	8.150^{b}	8.750^{a}
200 mg/kg	27.300^{b}	151.15^{b}	6.150^{c}	5.650^{c}
400 mg/kg	29.000^{ab}	142.70^{d}	11.300^{a}	7.000^{b}
600 mg/kg	30.400^{a}	145.55^{c}	8.400^{b}	4.800^{d}
SE±	0.639	0.343	0.087	0.112

Values are mean± SD

Means value(s) bearing no different superscript(s) in a column are not significantly different ($P \geq 0.05$) according to DMRT

SE ± Standard Error

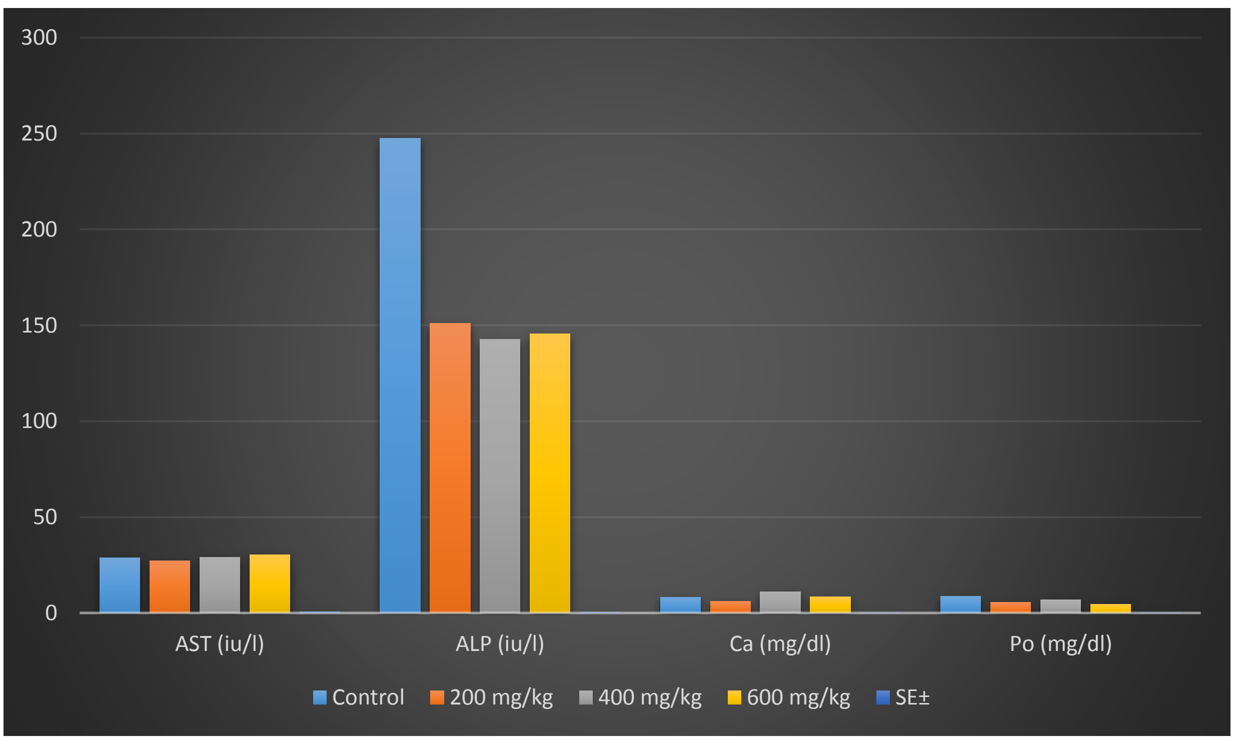


Fig.19: Effect of adding graded levels of Eucalyptus and Basil mixed essential oils on blood serum enzymes and minerals

Economical Appraisal:

Chicks purchase, feed cost, management, labor and electricity values were the main inputs considered. The total revenues obtained resemble the total selling values of the meat. Profitability ratio (1.147) of group of birds fed on 400 mg/kg mixed oils was the highest one, followed by birds fed on 200 mg/kg mixed oils, then those fed on 600 mg/kg mixed oils compared with group of birds fed on control diet.

Table (25): Economical appraisal for broiler chicks fed on graded levels Of Eucalyptus and Basil mixed essential oils:

Parameters	Control	200 mg/kg	400 mg/kg	600 mg/kg
Chick price	19.0	19.0	19.0	19.0
Diet cost	37.7	39.3	38.0	41.7
Labor	7.0	7.0	7.0	7.0
Total cost	63.7	65.3	64.0	67.7
Revenues	137.80	149.80	149.00	150.10
Profit	74.1	84.5	85.0	82.4
Profitability ratio	1.00	1.140	1.147	1.112

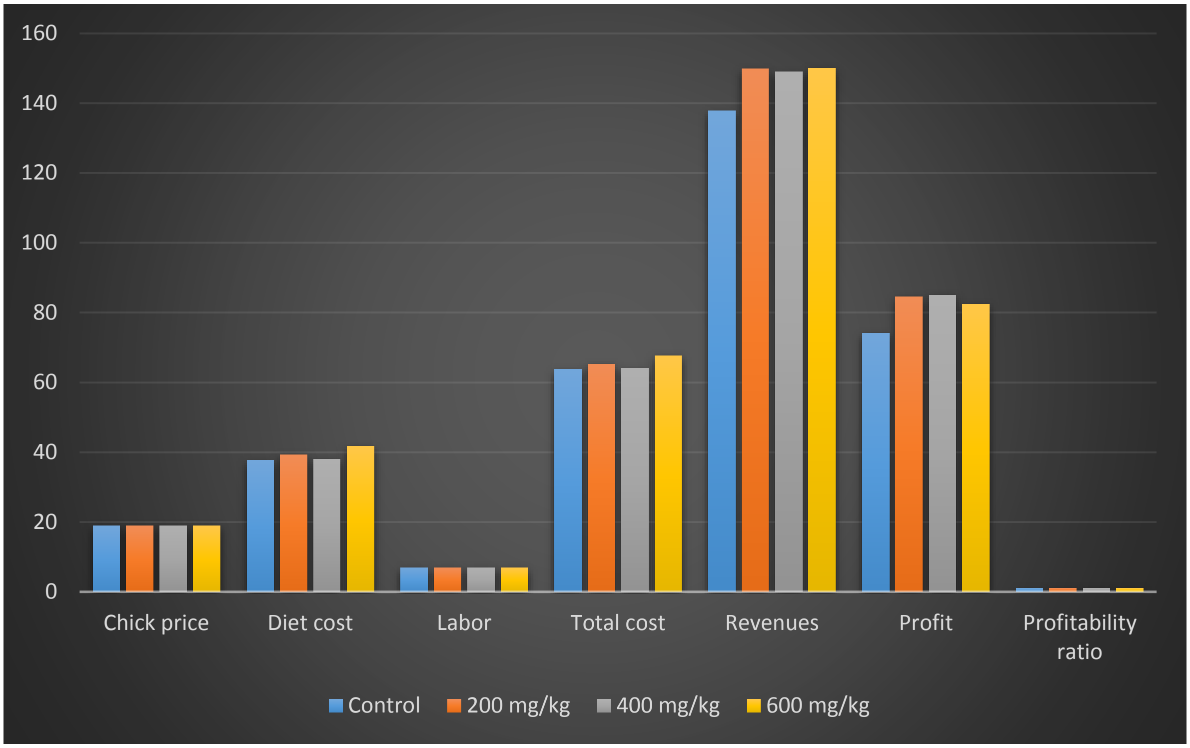


Fig.20: Economical appraisal for broiler chicks fed on graded levels of Eucalyptus and Basil mixed essential oils

4. 3 Experiment (3):

Response of broiler chicks fed on graded levels of Clove and Basil mixed essential oils:

The detected chemical composition of Clove Essential Oil, reported in Table (26), shows that 34 compounds were identified (Appendix 5), and that the component most present by far is Eugenol (46.54%) followed by Trans-Isoeugenol (11.77%), Isoeugenol (6.9%), Chavicol (3.24%), Kaempferol 3,7,4-trimethyl ether (2.59%), 2',3',4', Trimethoxyacetophenone (2.02%), a-Eudesmol (2.01).Whereas all the others are present in amounts lower than 2%.

Table (26): Chemical properties of Clove essential oil:

NO	RT(min)	Name	Area Sum%
1	10.40	Eugenol	46.54
2	11.56	Trans-Isoeugenol	11.77
3	9.60	Isoeugenol	6.9
4	9.33	Chavicol	3.24
5	17.68	Kaempferol 3,7,4-trimethyl ether	2.59
6	12.55	2',3',4', Trimethoxyacetophenone	2.02
7	12.42	a-Eudesmol	2.01

*source :(Regional Center for Food and Feed, Ministry of Agriculture, Egypt).

**RT=Retention Time (minute).

The performance results of broiler chicks fed on diets containing graded levels of Clove and Basil (CB) mixed essential oils were illustrated in table (27) and figure (21).

There were no significant ($P \geq 0.05$) differences for the parameters of performance among all tested groups. Except for feed conversion ratio. For body weight no significant ($P \geq 0.05$) differences, however group of chicks fed on 400 mg/kg mixed oils had numerically the highest weight compared with group fed on 200 mg/kg mixed oils, group fed on 600 mg/kg mixed oils and those fed on control diet which had the lowest weight. For feed intake no significant ($P \geq 0.05$) differences were noticed, but group of broilers fed on 200 mg/kg mixed oils had the highest compared with group fed on control diet, group fed on 400 mg/kg mixed oils and these fed on 600 mg/kg mixed oils which had the lowest. For body weight gain, also no significant ($P \geq 0.05$) differences, however group of broilers fed on 400 mg/kg mixed oils had the best weight gain compared with group fed on 200 mg/kg mixed oils, group fed on 600 mg/kg mixed oils and group fed on control diet which had the lowest weight. But for feed conversion ratio, results showed significantly increase with the increase of mixed oils in the diet, however group of birds fed on 400 mg/kg mixed oils showed the best value for feed conversion ratio.

Table (27): Effect of adding graded levels of Clove and Basil mixed essential oils on the performance of the broiler chicks:

Treatments	Initial body weight (g)	Final body weight (g)	Feed intake (g)	Body weight gain (g)	Feed conversion ratio
Control	187	1942.0	3428.7	1757.0	1.950^{a}
200 mg/kg	185	2129.3	3444.0	1944.3	1.770^{ab}
400 mg/kg	181	2145.0	3319.0	1960.0	1.700^{b}
600 mg/kg	183	2068.3	3301.0	1883.3	1.760^{ab}
SE± **Sig**	_	123.97 N.S	116.26 N.S	123.97 N.S	0.075

Values are mean± SD

Means value(s) bearing no different superscript(s) in a column are not significantly different (P≥0.05)

According to DMRT

SE ± Standard Er N.S = non-significant

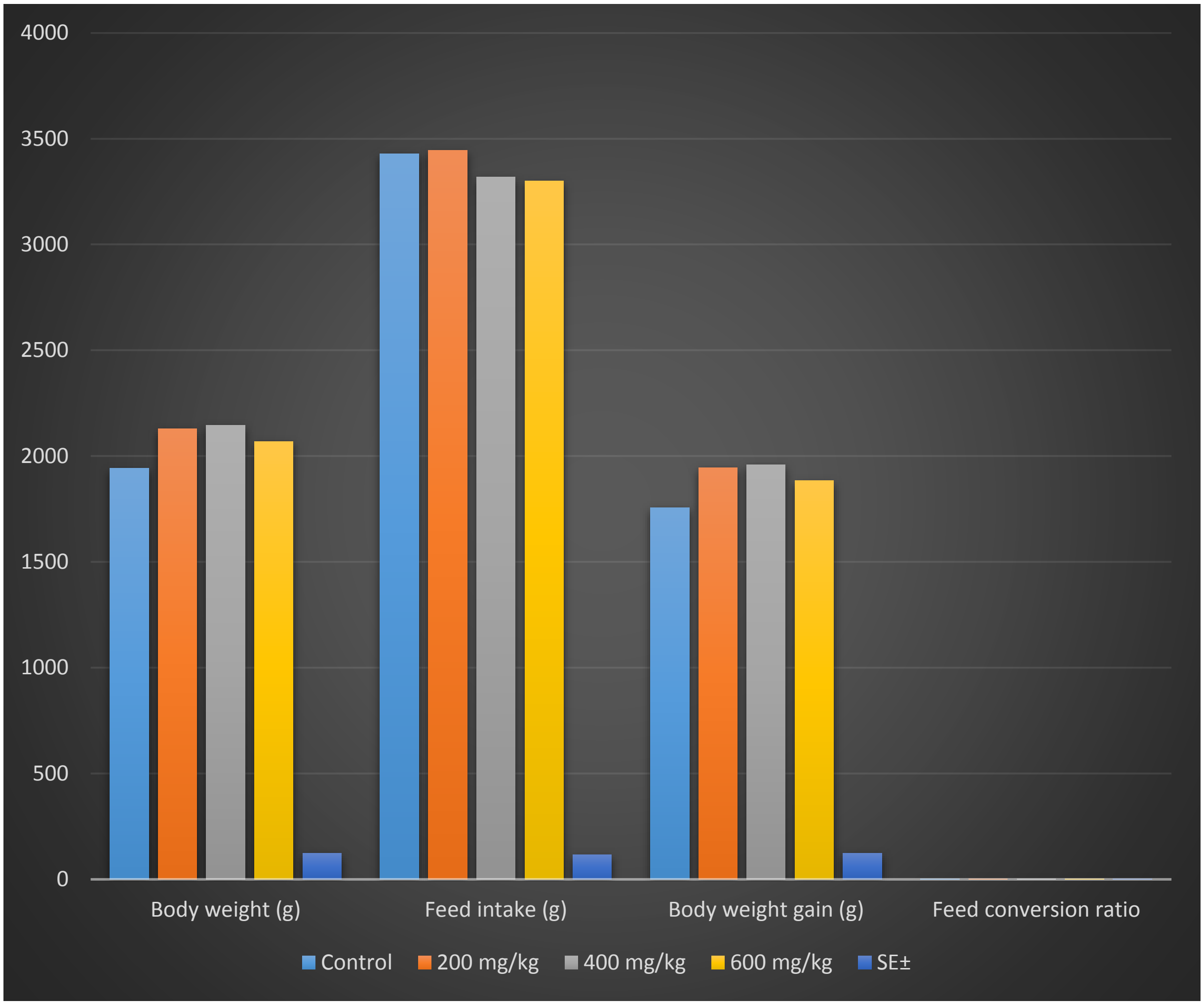


Fig.21: Effect of adding graded levels of Clove and Basil mixed essential oils on the performance of the broiler chicks

The results of adding graded levels of Clove and Basil mixed essential oils on dressing and giblets (liver, heart and gizzard) were showed in table (28) and figure (22).

Collected data for dressing percentage and giblets (liver, heart and gizzard) showed no significant ($P \geq 0.05$) differences among all tested groups.

The results of adding graded levels of Clove and Basil mixed essential oils on non-carcass were showed in table (29) and figure (23).

No significant ($P \geq 0.05$) differences were observed among all tested groups for non-carcass.

Table (28): Effect of adding graded levels of Clove and Basil mixed essential oils on dressing and giblets (Liver, heart and gizzard):

Treatments	Dressing%	Liver%	Heart%	Gizzard%
Control	70.310	2.047	0.510	1.547
200 mg/kg	70.680	2.230	0.603	1.437
400 mg/kg	68.320	2.037	0.537	1.390
600 mg/kg	70.457	1.803	0.503	1.340
SE±	1.014	0.179	0.090	0.210
Sig	N.S	N.S	N.S	N.S

Values are mean± SD, Means value(s) bearing no different superscript(s) in a column are not significantly different (P≥0.05) according to DMRT, SE ± Standard Error N.S = non-significant

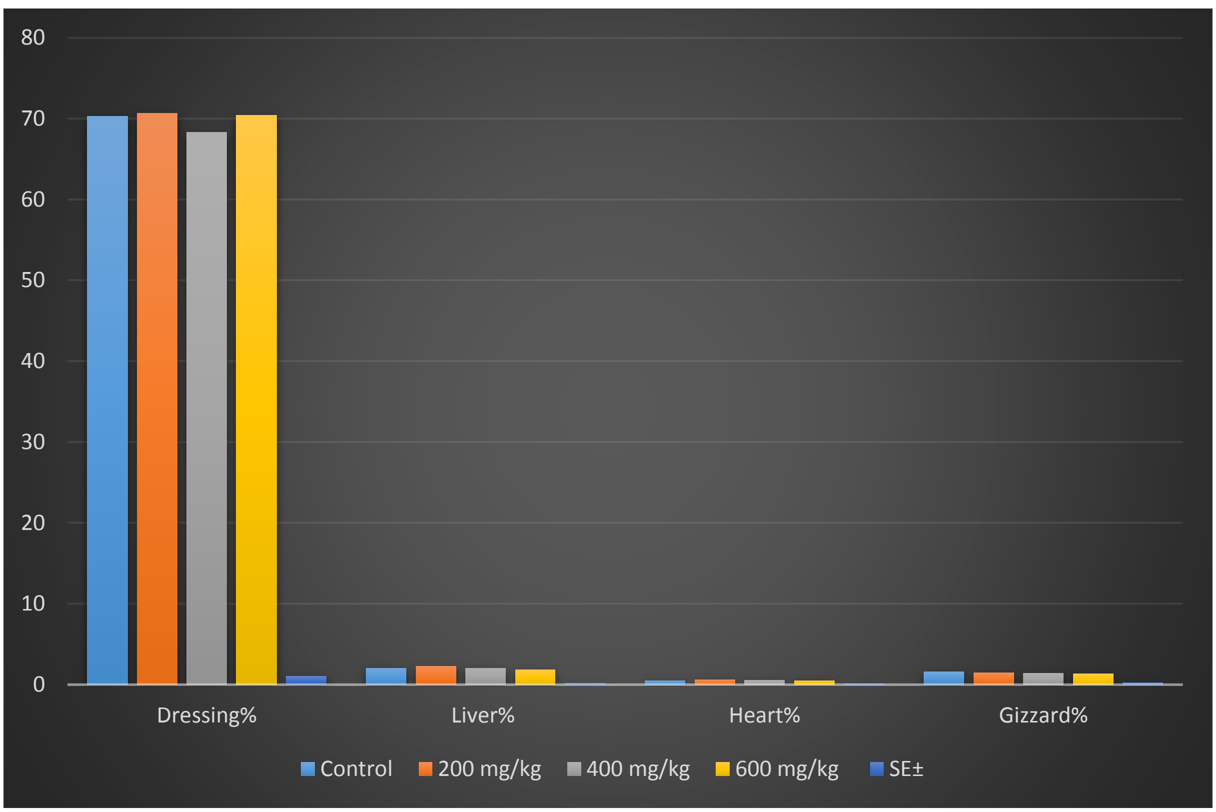


Fig.22: Effect of adding graded levels of Clove and Basil mixed essential oils on dressing and giblets (Liver, heart and gizzard)

Table (29): Effect of adding graded levels of Clove and Basil mixed essential oils on non-carcass:

Treatments	Head %	Legs %	Lung %	Kidney %	Abdominal fat%	Intestine weight%	Intestine length%	Back %	Wing %	Neck %
Control	2.553	3.620	0.727	0.373	1.067	3.800	178.33	19.693	10.467	5.193
200 mg/kg	2.037	3.587	0.687	0.513	1.220	3.777	180.00	19.867	10.833	4.757
400 mg/kg	2.103	3.580	0.727	0.643	0.990	3.617	177.33	19.100	9.300	4.593
600 mg/kg	2.500	3.590	0.603	0.540	0.807	3.677	176.67	21.333	10.700	4.650
SE±	0.184	0.253	0.115	0.121	0.209	0.305	6.272	1.108	0.719	0.399
Sig	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S

Values are mean± SD

Means value(s) bearing no different superscript(s) in a column are not significantly different ($P \geq 0.05$) according to DMRT

SE ± Standard Error N.S = non-significant

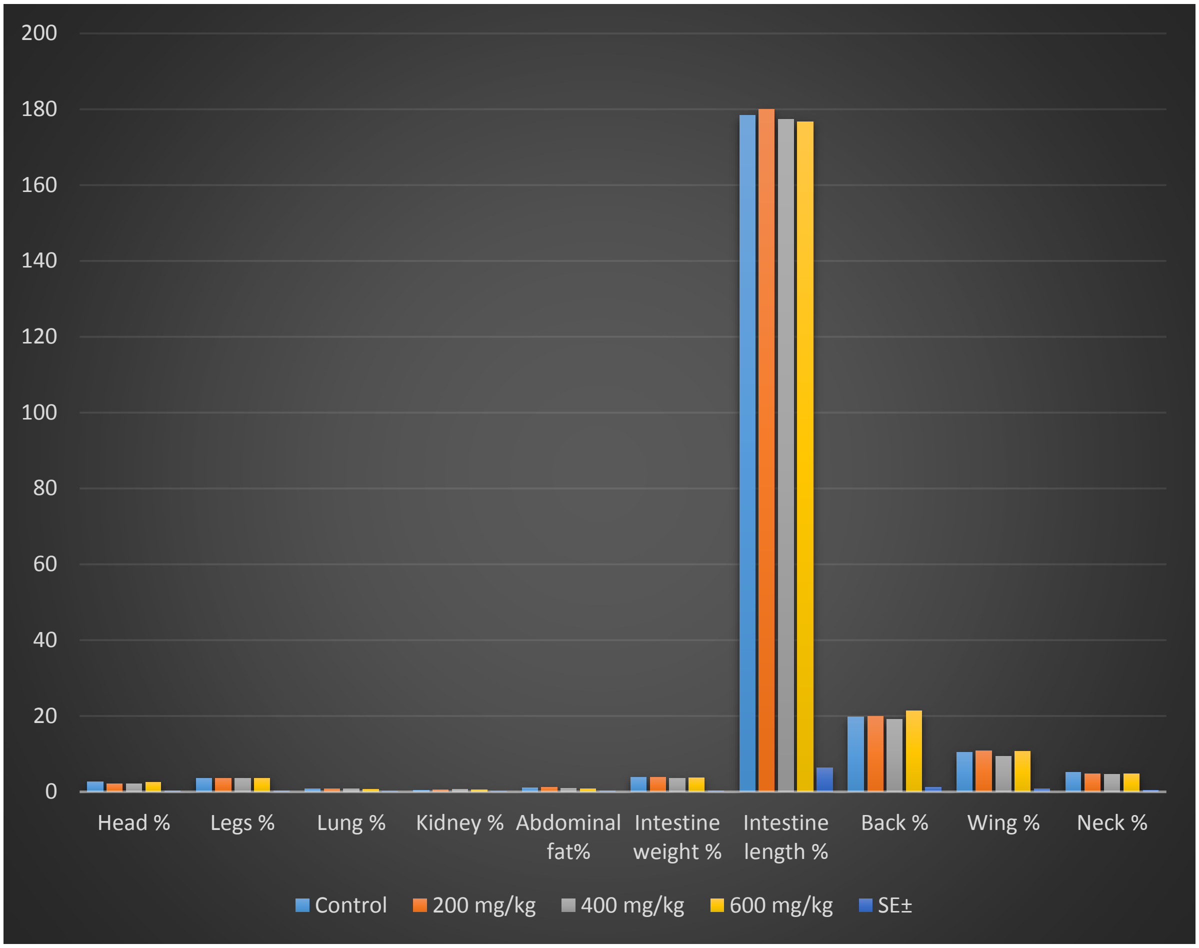


Fig.23: Effect of adding graded levels of Clove and Basil mixed essential oils on non-carcass

The results of adding graded levels of Clove and Basil mixed essential oils on commercial cuts were illustrated in table (30) and figure (24), their meat values showed in table (31) and figure (25).

Results showed no significant ($P \geq 0.05$) differences among all tested groups for commercial cuts and their meat percentages.

Table (30): Effect of adding graded levels of Clove and Basil Mixed essential oils on commercial cuts:

Treatments	Breast%	Drumstick%	Thigh%
Control	39.460	11.730	15.067
200 mg/kg	37.613	11.717	13.487
400 mg/kg	37.633	12.973	14.493
600 mg/kg	36.853	12.157	13.897
SE±	1.427	1.254	1.207
Sig	N,S	N.S	N.S

Values are mean± SD Means value(s) bearing no different superscript(s) in a column are not significantly different (P≥0.05) according to DMRT SE ± Standard Error N.S = non-significant

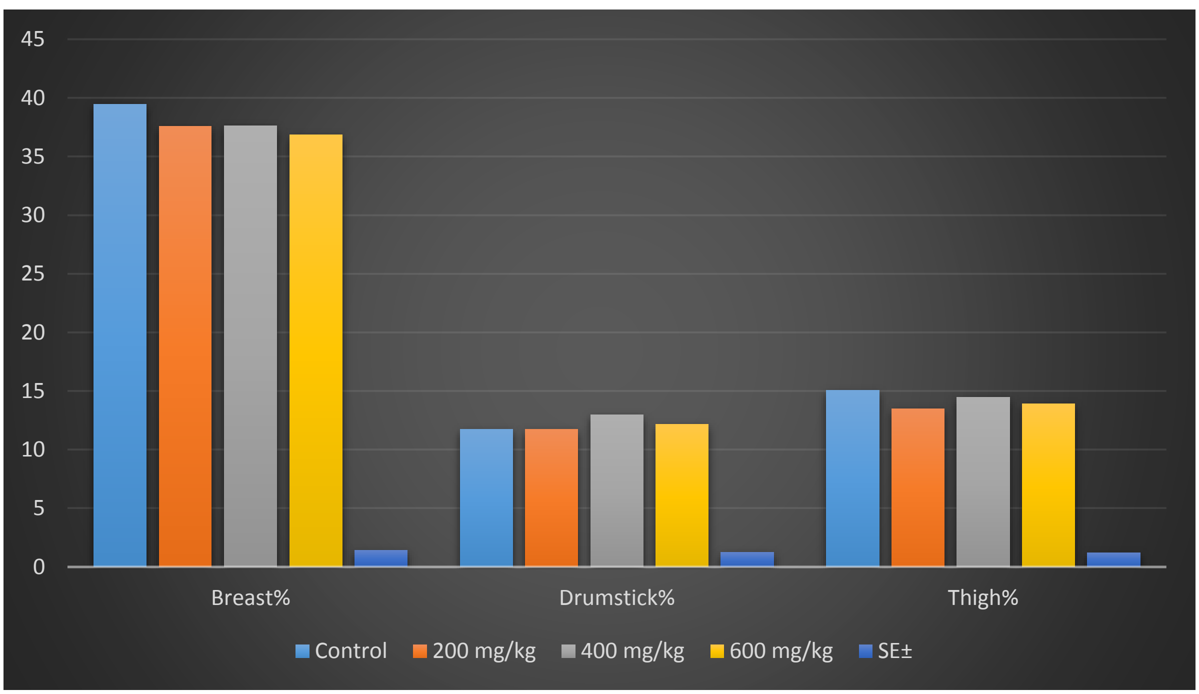


Fig.24: Effect of adding graded levels of Clove and Basil mixed essential oils on commercial cuts

Table (31): Effect of adding graded levels of Clove and Basil mixed essential oils on commercial cuts meat:

Treatments	Breast meat%	Drumstick meat%	Thigh meat%
Control	85.150	72.533	84.783
200 mg/kg	84.967	70.067	84.467
400 mg/kg	87.400	74.400	86.567
600 mg/kg	86.167	70.567	84.600
SE±	2.639	2.057	2.314
Sig	N.S	N.S	N.S

Values are mean± SD, Means value(s) bearing no different superscript(s) in a column are not significantly different (P≥0.05) according to DMRT SE ± Standard Error N.S = non-significant

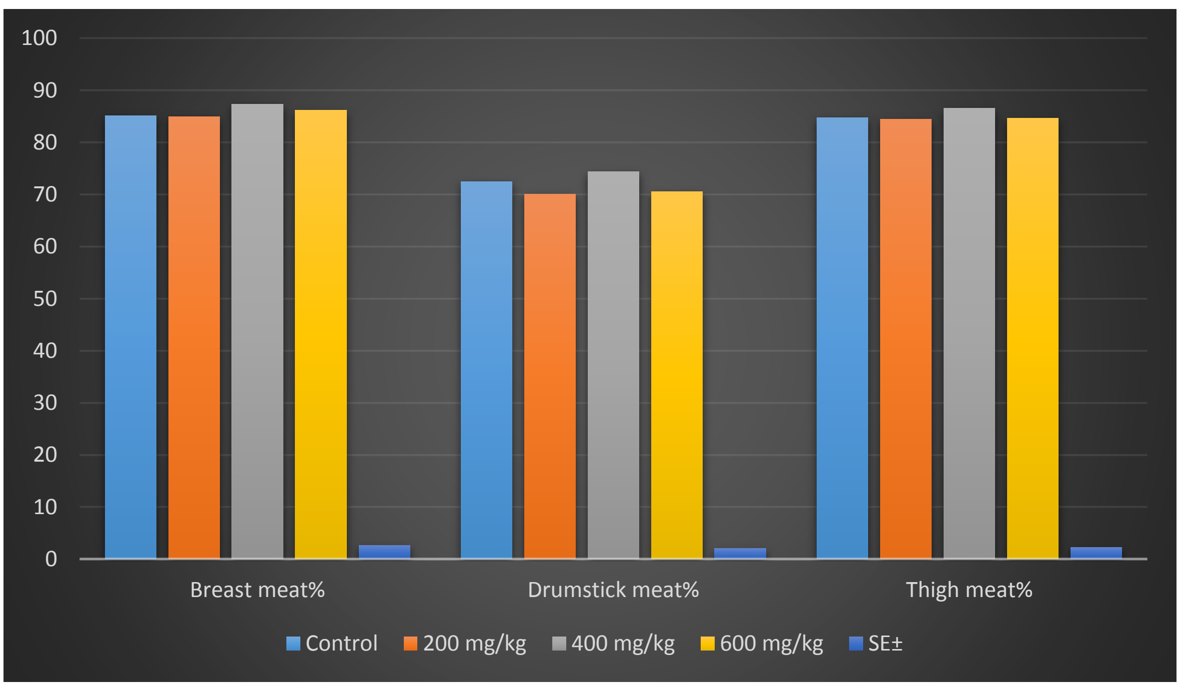


Fig.25: Effect of adding graded levels of Clove and Basil mixed essential oils on commercial cuts meat

The results of sensory evaluation of chicks' meat fed on graded levels of Clove and Basil mixed essential oils were showed in table (32) and figure (26).

Results obtained showed significant ($P \leq 0.05$) differences between all treatments for all parameters. Juiciness showed significant decrease with the addition of mixed essential oils, the same results were recorded for color and tenderness, but not for flavor. Control group recorded moderately in the above parameters, whowever all scores given were within normal.

Table (32): Sensory evaluation of chick's meat fed on graded levels Of Clove and Basil mixed essential oils:

Treatments	Juiciness	Color	Flavor	Tenderness
Control	6.1300[a]	6.060[a]	5.450[c]	6.110[a]
200 mg/kg	5.480[c]	5.740[c]	5.840[a]	5.600[c]
400 mg/kg	5.850[b]	5.940[b]	5.680[b]	6.010[a]
600 mg/kg	5.820[b]	6.070[a]	5.870[a]	5.780[b]
SE±	0.017	0.035	0.027	0.055

Values are mean± SD, Any two mean value(s) bearing different superscript(s) in a column are significantly different (P≥0.05) according to DMRT, SE± Standard Error

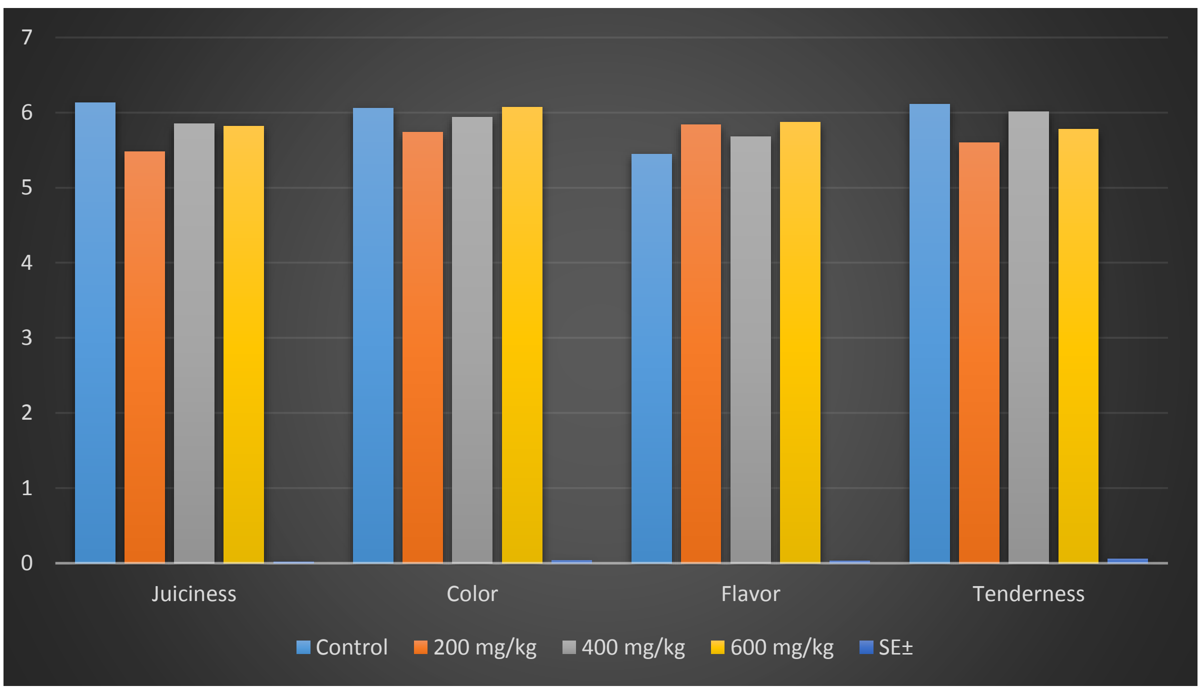


Fig.26: Sensory evaluation of chick's meat fed on graded levels of Clove and Basil mixed essential oils

The results of adding graded levels of Clove and Basil mixed essential oils on meat chemical composition were showed in table (33) and figure (27).

For moisture, no significant ($P \geq 0.05$) difference between group of chicks fed on 200 mg/kg mixed oils and group fed on 400 mg/kg mixed oils compared with group fed on 600 mg/kg mixed oils and control group which had the lowest. For dry matter no significant ($P \geq 0.05$) difference between group of chicks fed on 200 mg/kg mixed oils and group fed on 400 mg/kg mixed oils compared with control group which had the highest and group fed on 600 mg/kg mixed oils which had the lowest. Whereas for ash no significant ($P \geq 0.05$) differences were observed among all tested groups. For crude protein analysis also revealed no significant ($P \geq 0.05$) differences among experimental groups, except group fed on 400 mg/kg mixed oils showed slightly significant decrease. For ether extract analysis showed significant decrease with the adding of mixed essential oils, so control group showed significantly the highest level, while no significant ($P \geq 0.05$) differences observed among tested groups.

Table (33): Effect of adding graded levels of Clove and Basil mixed essential oils on meat chemical composition:

Treatments	Moisture%	Dry-matter%	Ash%	Crude protein%	Ether extract%
Control	73.233[b]	26.767[a]	1.200[a]	22.583[a]	1.377[a]
200 mg/kg	74.950[ab]	25.050[ab]	1.133[a]	22.140[a]	0.800[b]
400 mg/kg	74.400[ab]	25.600[ab]	1.250[a]	21.270[b]	0.800[b]
600 mg/kg	76.250[a]	23.750[b]	1.100[a]	22.460[a]	0.450[b]
SE±	0.645	0.645	0.069	0.161	0.134

Values are mean± SD, Means value(s) bearing no different superscript(s) in a column are not significantly different (P≥0.05) according to DMRT, SE ± Standard Error

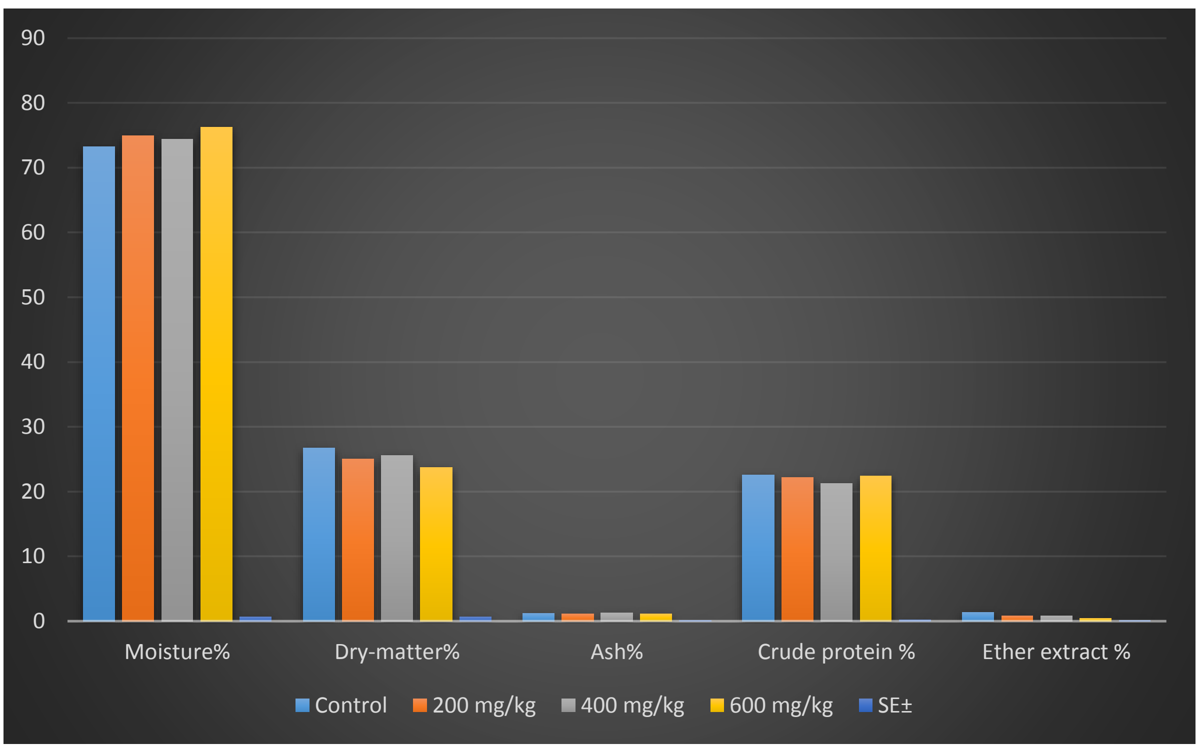


Fig.27: Effect of adding graded levels of Clove and Basil mixed essential oils on meat chemical composition

The results of adding graded levels of Clove and Basil mixed essential oils on blood serum metabolites were showed in table (34) and figure (28).

For glucose, no significant (P≥0.05) difference between group of chicks fed on control diet and group of chicks fed 200 mg/kg mixed oils compared with group of chicks fed on 600 mg/kg mixed oils and group of chicks fed on 400 mg/kg mixed oils which had the lowest level, and for triglyceride no significant (P≥0.05) difference between group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils compared with group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on control diet which had the lowest level, also for total protein no significant (P≥0.05) difference between group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils compared with group of chicks fed on control diet which had the highest and group of chicks fed on 200 mg/kg mixed oils which had the lowest, and for albumin there were no significant (P≥0.05) difference between group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils compared with group of chicks fed on control diet which had the highest level and group of chicks fed on 400 mg/kg mixed oils which had the lowest, also for cholesterol no significant (P≥0.05) differences were observed between group of chicks fed on control diet, group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils compared with group of chicks fed on 200 mg/kg mixed oils which had the lowest level, for HDL no significant (P≥0.05) difference between group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils compared with group of chicks fed on control diet and group of chicks fed on 400 mg/kg mixed oils which had the highest, but for LDL there were significant (P≤0.05) differences among all tested groups, group of chicks fed on 200 mg/kg mixed oils had the lowest level compared with group of chicks fed on control diet and group of chicks fed on 600 mg/kg mixed oils, while group of chicks fed on 400 mg/kg mixed oils had the highest, for urea no significant ($P \geq 0.05$) difference between group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils compared with group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on control diet which had the highest, and for uric acid no significant ($P \geq 0.05$) differences between group of chicks fed on 200 mg/kg mixed oils, group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils compared with group of chicks fed on control diet which had the lowest, finally for creatinine no significant ($P \geq 0.05$) difference between group of chicks fed on control diet and group of chicks fed on 200 mg/kg mixed oils compared with group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils which had the highest.

Table (34): Effect of adding graded levels of Clove and Basil mixed essential oils on blood serum Metabolites:

Treatments	Glucose (mg\dl)	Tri-gly Ceride (mg\dl)	Protein (g/dl)	Alb. (g/dl)	Cholest. (mg/dl)	Chol. HDL (mg/dl)	Chol. LDL (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Creatine (mg/dl)
Control	220.50^{a}	43.500^{c}	3.950^{a}	2.050^{a}	124.50^{a}	130.50^{b}	22.500^{c}	7.100^{a}	3.450^{b}	2.110^{c}
200 mg/kg	222.00^{a}	51.500^{b}	2.300^{c}	1.950^{ab}	114.00^{b}	126.70^{bc}	20.000^{d}	3.500^{b}	7.400^{a}	2.160^{bc}
400 mg/kg	171.00^{c}	61.500^{a}	2.950^{b}	1.650^{c}	125.00^{a}	140.05^{a}	36.500^{a}	2.100^{c}	7.400^{a}	2.190^{b}
600 mg/kg	212.50^{b}	49.500^{b}	3.000^{b}	1.850^{b}	122.50^{a}	124.50^{c}	25.500^{b}	2.050^{c}	7.200^{a}	2.350^{a}
SE±	1.384	1.225	0.050	0.041	0.957	1.190	0.540	0.213	0.179	0.016

Values are mean± SD

Any two mean value(s) bearing different superscript(s) in a column are significantly different ($P \geq 0.05$) according to DMRT

SE ± Standard Error

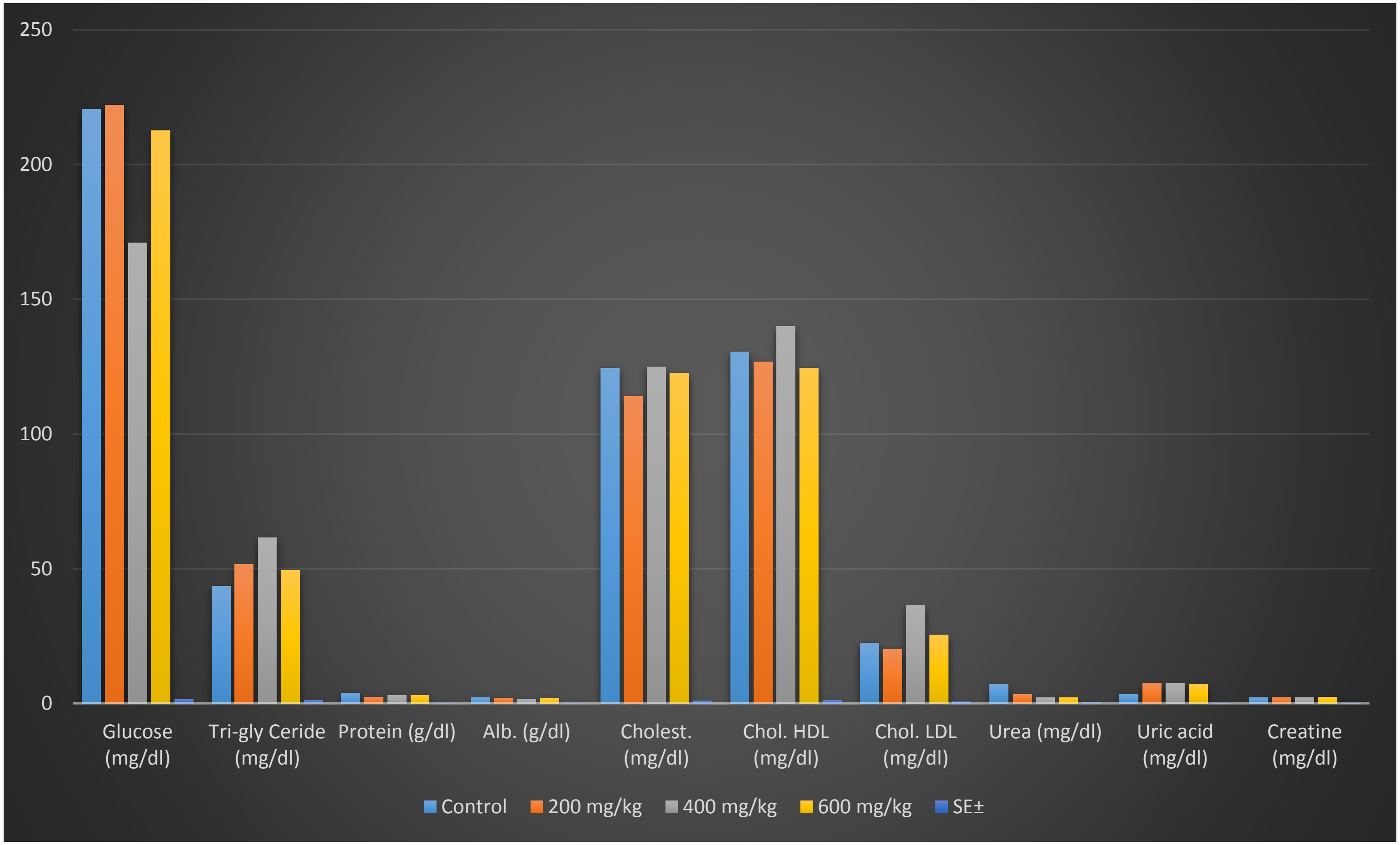


Fig.28: Effect of adding graded levels of Clove and Basil mixed essential oils on blood serum metabolites

The results of adding graded levels of Clove and Basil mixed essential oils on serum enzymes and minerals were illustrated in table (35) and figure (29).

For Aspartate Amino Transferase (AST) no significant ($P \geq 0.05$) differences among all tested groups, however group of chicks fed on 400 mg/kg mixed oils had the lowest level compared with other tested groups, but for Alkaline Phosphatase (ALP) there were significant ($P \leq 0.05$) differences among all tested groups, control group had the highest level, while the addition of mixed oils had decreased significant values.

For Ca treated groups showed significantly high concentration compared with control group, in the contrary, the addition of mixed oils significantly decreased the P concentration, and therefore control group showed significantly the highest level while group fed on 600 mg/kg mixed oils revealed significantly the lowest level.

Table (35): Effect of adding graded levels of Clove and Basil mixed essential oils on serum enzymes and minerals:

Treatments	AST (iu/l)	ALP (iu/l)	Ca (mg/dl)	Po (mg/dl)
Control	28.950^{a}	247.50^{a}	8.150^{b}	8.750^{a}
200 mg/kg	29.150^{a}	216.50^{d}	8.450^{a}	6.750^{c}
400 mg/kg	28.000^{a}	235.50^{c}	8.500^{a}	7.600^{b}
600 mg/kg	29.150^{a}	241.00^{b}	8.450^{a}	4.950^{d}
SE±	0.639	0.540	0.079	0.035

Values are mean± SD

Means value(s) bearing no different superscript(s) in a column are not significantly different

(P≥0.05) according to DMRT

SE ± Standard Error

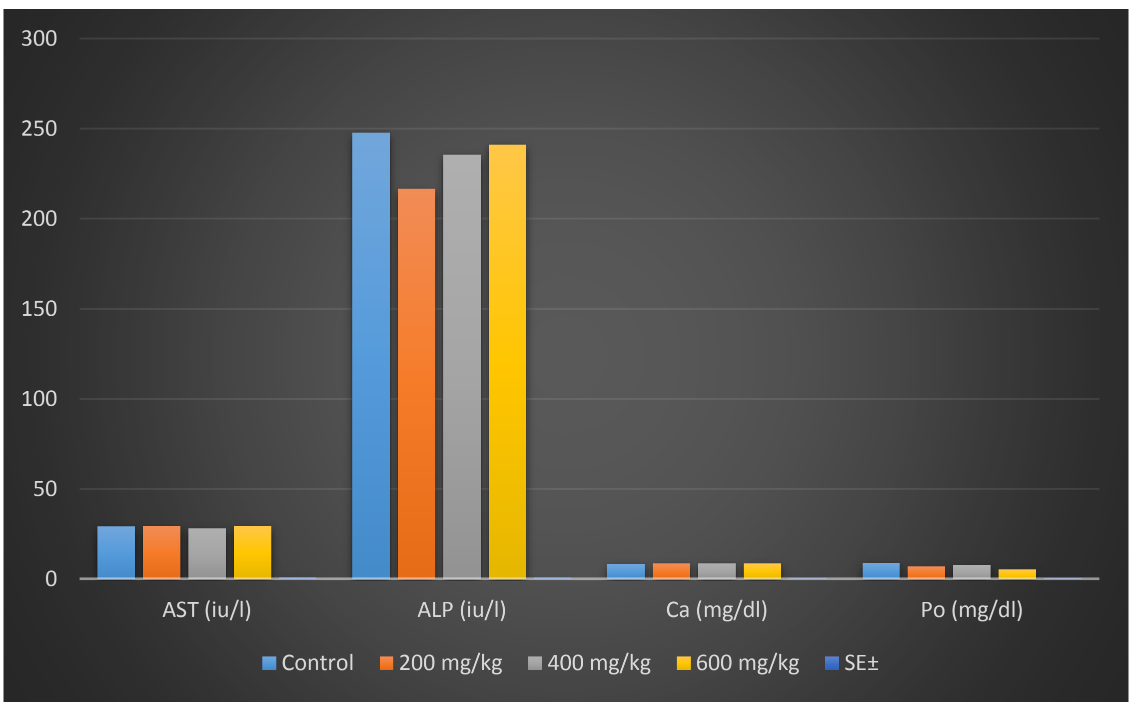


Fig.29: Effect of adding graded levels of Clove and Basil mixed essential oils on serum enzymes and minerals

Economical Appraisal:

Chicks purchase, feed cost, management, labor and electricity values were the main inputs considered. The total revenues obtained resemble the total selling values of the meat. Profitability ratio (1.222) for group of birds fed on 200 mg/kg mixed oils was the highest one, followed by group of birds fed on 600 mg/kg mixed oils, then those fed on 400 mg/kg mixed oils, compared with group of birds fed on control diet.

Table (36): Economical appraisal for broiler chicks fed on graded levels of Clove and Basil mixed essential oils:

Parameters	Control	200 mg/kg	400 mg/kg	600 mg/kg
Chick price	19.0	19.0	19.0	19.0
Diet cost	37.7	41.4	43.5	46.8
Labor	7.0	7.0	7.0	7.0
Total cost	63.7	67.4	69.5	72.8
Revenues	137.8	158.0	144.2	156.8
Profit	74.1	90.6	74.7	84.0
Profit ability ratio	1	1.222	1.008	1.134

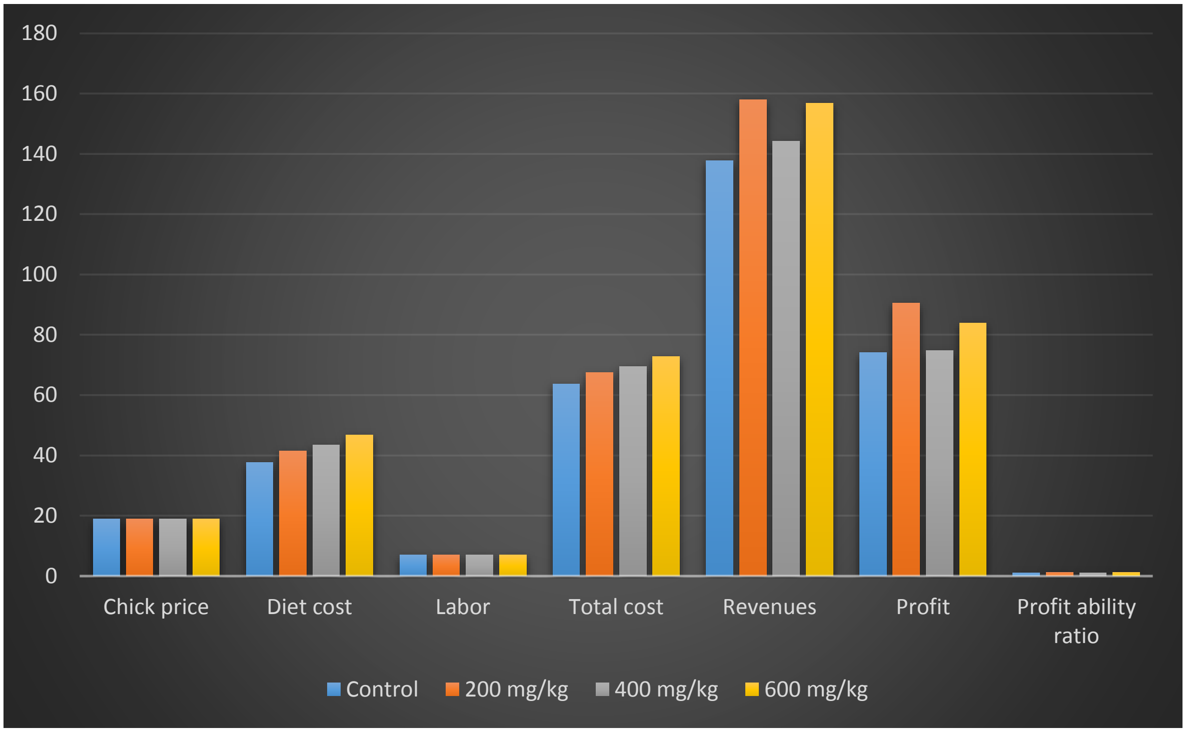


Fig.30: Economical appraisal for broiler chicks fed on graded levels of Clove and Basil mixed essential oils

4. 4 Experiment (4):

Response of broiler chicks fed on graded levels of Eucalyptus, Clove and Basil mixed essential oils:

The performance results of broiler chicks fed on diets containing graded levels of Eucalyptus, Clove and Basil mixed essential oils were illustrated in table (37) and figure (31).

Results showed no significant (P≥0.05) differences for all parameters. For body weight group of chicks fed on 400 mg/kg mixed oils had the highest weight, and control group had the lowest, compared with group fed on 200 mg/kg mixed oils and group fed on 600 mg/kg mixed oils, and for feed intake no significant (P≥0.05) differences between all tested groups, but group of chicks fed on 200 mg/kg mixed oils had the highest, and group fed on 600 mg/kg mixed oils had the lowest, compared with control group and group fed on 4oo mg/kg mixed oils, also for body weight gain no significant (P≥0.05) differences between all tested groups, but group of chicks fed on 400 mg/kg mixed oils had the highest gain and control group had the lowest, compared with group of chicks fed on 200 mg/kg mixed oils and group fed on 600 mg/kg mixed oils. Feed conversion ratio decreased numerically with increase level of mixed essential oils in the diet.

Table (37): Effect of adding graded levels of Eucalyptus, Clove and Basil mixed essential oils on the performance of the broiler chicks:

Treatments	Initial body weight (g)	Final body weight (g)	Feed intake (g)	Body weight gain (g)	Feed conversion ratio
Control	187	1942.0	3428.7	1757.0	1.950
200 mg/kg	185	1979.7	3437.7	1794.7	1.913
400 mg/kg	180	2074.7	3399.0	1889.7	1.813
600 mg/kg	184	2045.7	3342.3	1860.7	1.810
SE±	_	137.88	143.87	137.88	0.135
Sig		N.S	N.S	N.S	N.S

Values are mean± SD, Means value(s) bearing no different superscript(s) in a column are not significantly different (P≥0.05) according to DMRT, SE± standard Error N.S = non-significant

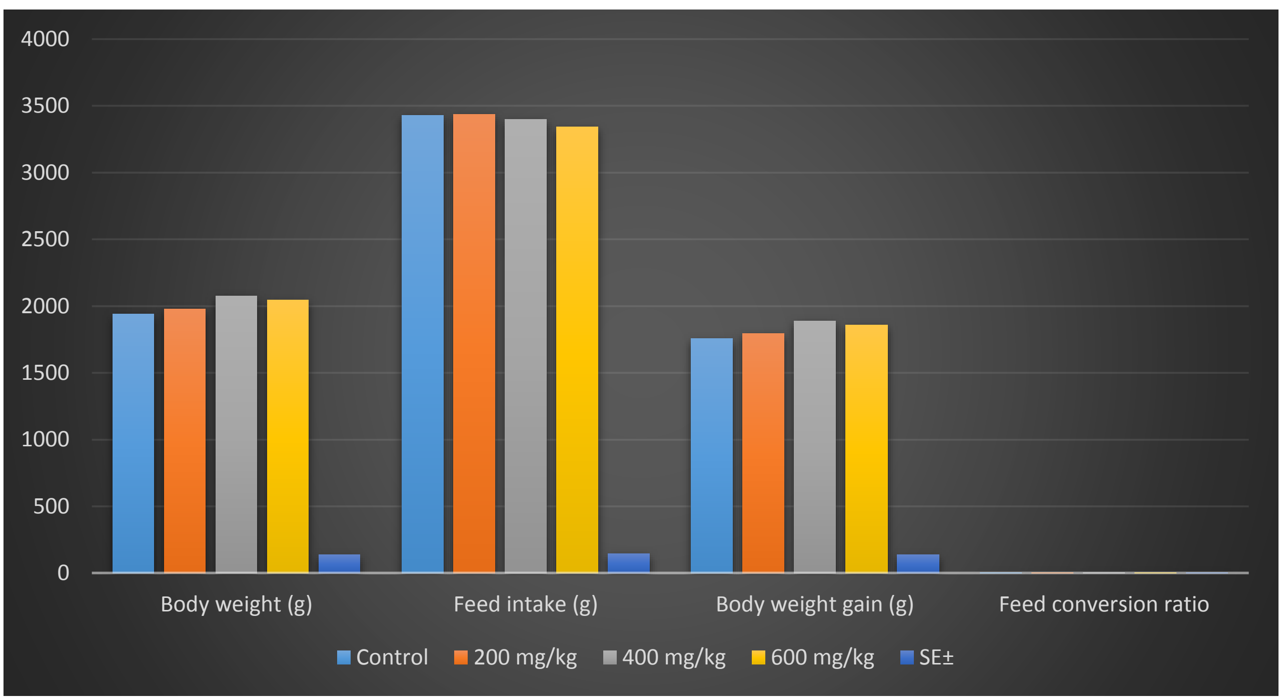


Fig.31: Effect of adding graded levels of Eucalyptus, Clove and Basil mixed essential oils on the performance of the broiler chicks

The results of adding graded levels of Eucalyptus, Clove and Basil mixed essential oils on dressing and giblets were illustrated in table (38) and figure (32).

For dressing percentage no significant ($P \geq 0.05$) differences between all tested groups, however group of chicks fed on 200 mg/kg mixed oils had the highest and group of chicks fed on 400 mg/kg mixed oils had the lowest, compared with group of chicks fed on control diet and group of chicks fed on 600 mg/kg mixed oils, on the other hands no significant ($P \geq 0.05$) differences between all tested groups for giblets (liver, heart and gizzard).

Table (38): Effect of adding graded levels of Eucalyptus, Clove and Basil Mixed essential oils on dressing and giblets (liver, heart, gizzard):

Treatments	Dressing%	Liver%	Heart%	Gizzard%
Control	70.310	2.047	0.510	1.547
200 mg/kg	71.653	1.983	0.473	1.427
400 mg/kg	64.423	2.340	0.597	1.547
600 mg/kg	69.660	2.247	0.630	1.490
SE± **Sig**	3.495 N.S	0.239 N.S	0.082 N.S	0.114 N.S

Values are mean± SD, Means value(s) bearing no different superscript(s) in a column are not significantly different (P≥0.05) according to DMRT, SE ± Standard Error N.S = non-significant

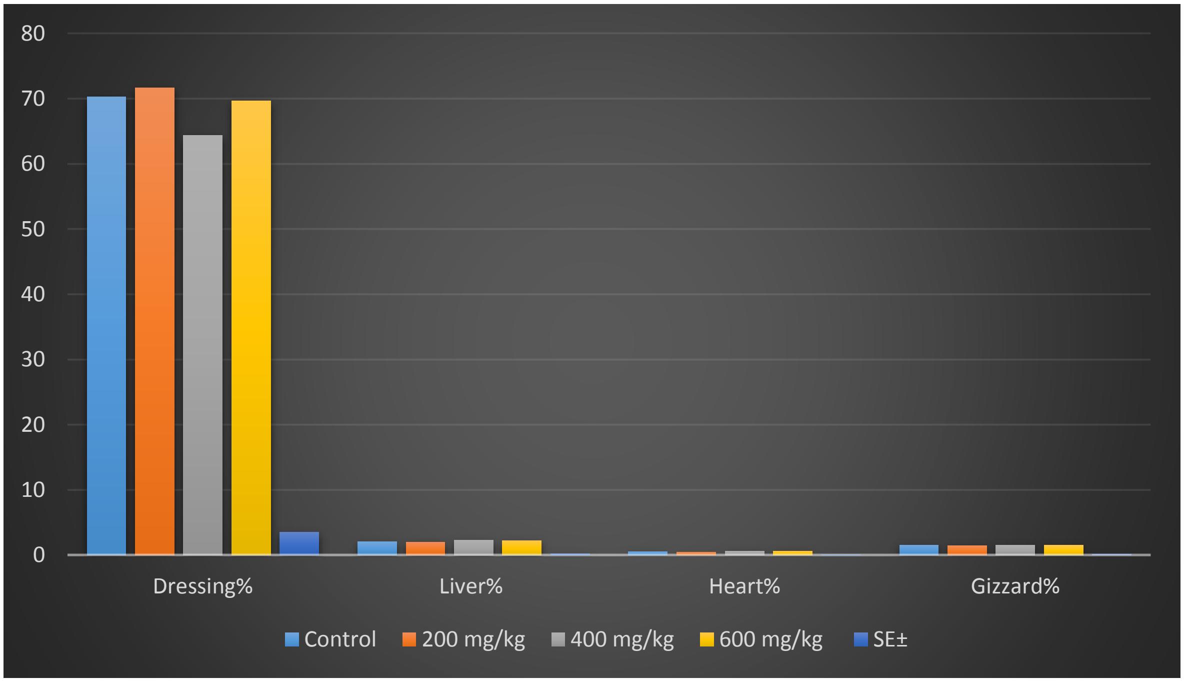


Fig.32: Effect of adding graded levels of Eucalyptus, Clove and Basil mixed essential oils on dressing and giblets (liver, heart and gizzard)

The results of adding graded levels of Eucalyptus, Clove and Basil mixed essential oils on non-carcass were showed in table (39) and figure (33).

There were no significant ($P \geq 0.05$) differences observed between all tested groups for non-carcass percentages.

Table (39): Effect of adding graded levels of Eucalyptus, Clove and Basil mixed essential oils on Non-carcass percentages:

Treatments	Head %	Legs %	Lung %	Kidney %	Abdominal fat%	Intestine weight %	Intestine length%	Back %	Wing %	Neck %
Control	2.553	3.620	0.727	0.373	1.067	3.800	178.33	19.693	10.467	5.193
200 mg/kg	2.363	3.887	0.473	0.427	0.757	3.500	184.67	20.533	10.200	4.730
400 mg/kg	2.477	3.873	0.583	0.463	1.187	3.470	183.00	17.733	10.533	4.250
600 mg/kg	2.073	3.803	0.643	0.477	0.957	3.730	172.33	20.300	10.400	4.270
SE± **Sig**	0.253 N.S	0.397 N.S	0.116 N.S	0.058 N.S	0.155 N.S	0.296 N.S	7.394 N.S	0.916 N.S	0.559 N.S	0.353 N.S

Values are mean± SD

Means value(s) bearing no different superscript(s) in a column are not significantly different

($P \geq 0.05$) according to DMRT

SE ± Standard Error N.S = non-significant

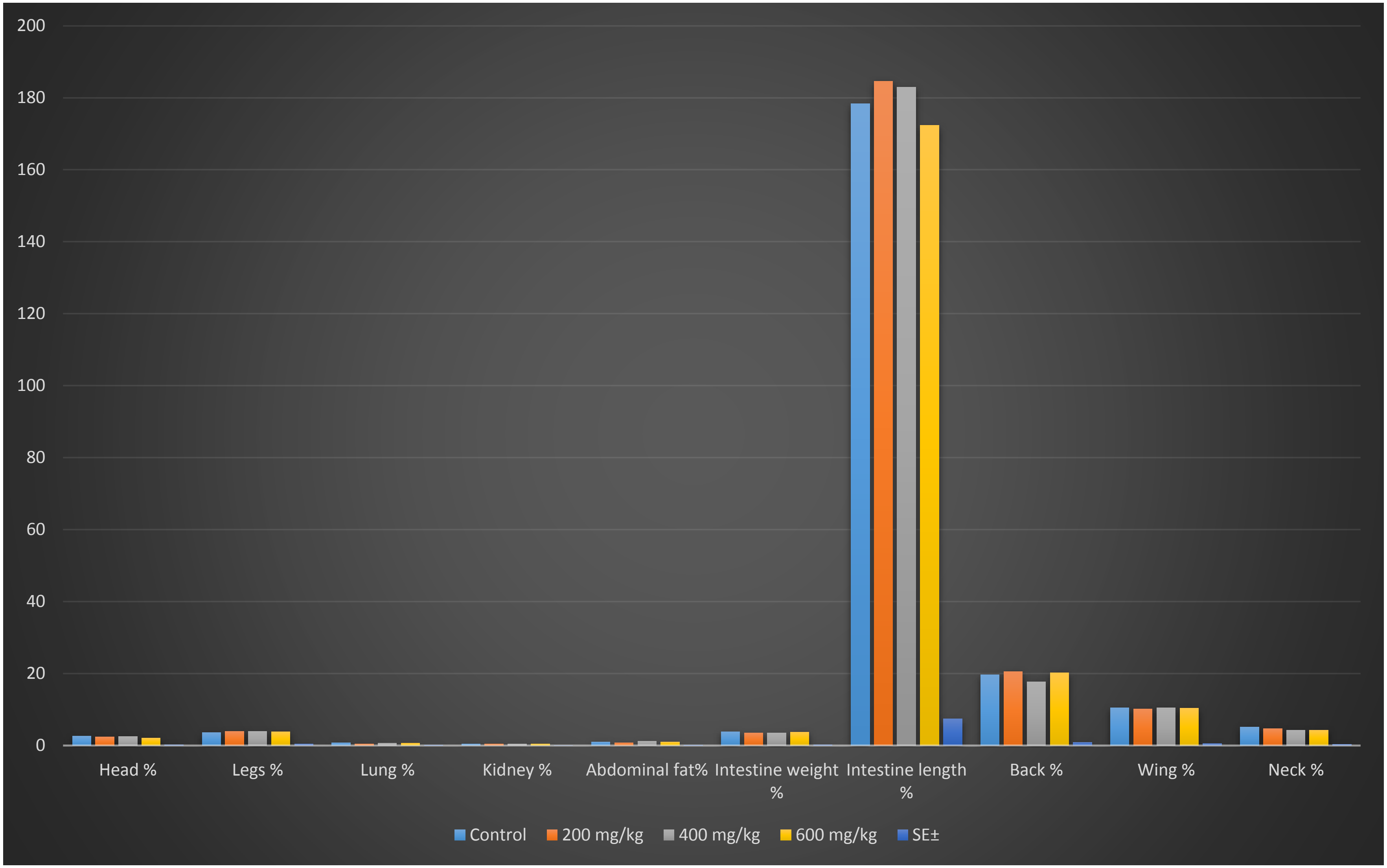


Fig.33: Effect of adding graded levels of Eucalyptus, Clove and Basil mixed essential oils on non-carcass percentages

The results of feeding graded levels of Eucalyptus, Clove and Basil mixed essential oils on commercial cuts were showed in table (40) and figure (34), their meat values were showed in table (41) and figure (35).

No significant ($P \geq 0.05$) differences between all tested groups for commercial cuts, and their meat percentages.

Table (40): Effect of adding graded levels of Eucalyptus, Clove And Basil mixed essential oils on commercial cuts:

Treatments	Breast%	Drumstick%	Thigh%
Control	39.460	11.730	15.067
200 mg/kg	38.250	12.417	13.260
400 mg/kg	36.220	13.170	14.510
600 mg/kg	36.463	12.867	13.573
SE±	1.030	1.266	1.396
Sig	N.S	N.S	N.S

Values are mean± SD, Means value(s) bearing no different superscript(s) in a column are not Significantly different (P≥0.05) according to DMRT, SE ± Standard Error N.S= non-significant

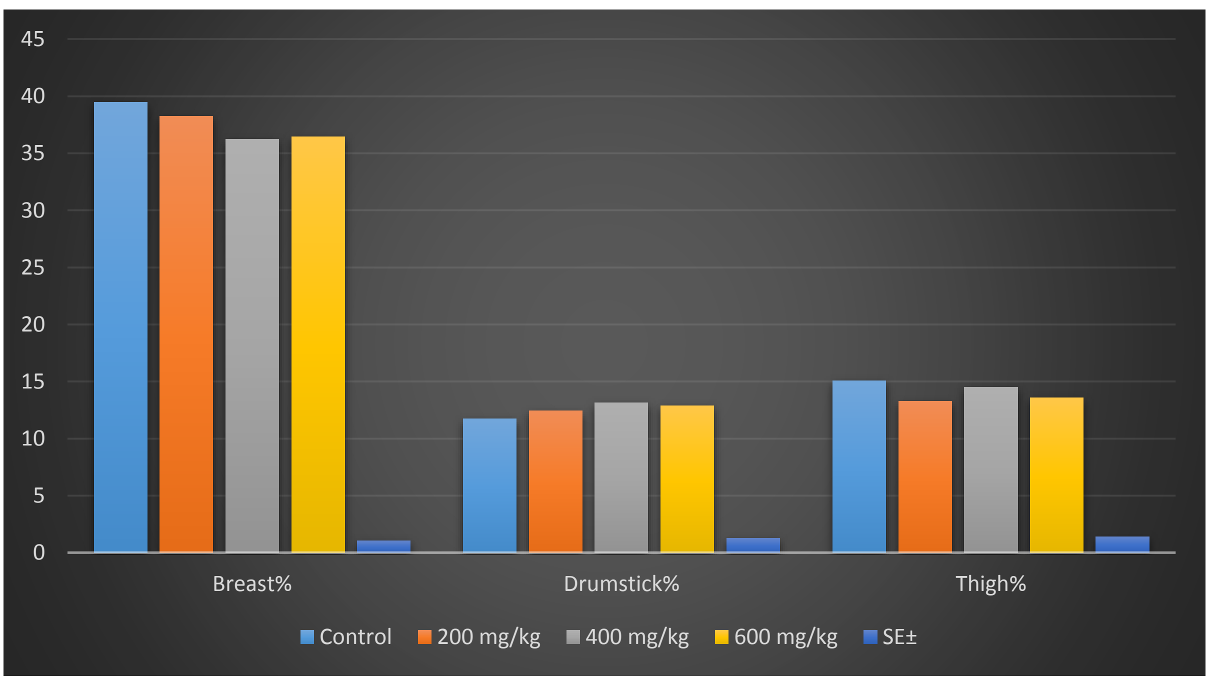


Fig.34: Effect of adding graded levels of Eucalyptus, Clove and Basil mixed Essential oils on commercial cuts

Table (41): Effect of adding graded levels of Eucalyptus, Clove and Basil mixed essential oils on commercial cuts meat percentages:

Treatments	Breast meat %	Drumstick meat%	Thigh meat %
Control	85.150[a]	72.533[a]	84.783[a]
200 mg/kg	85.533[a]	75.200[a]	83.767[a]
400 mg/kg	85.833[a]	69.467[a]	83.400[a]
600 mg/kg	85.133[a]	68.733[a]	81.167[a]
SE±	2.309	3.320	1.892

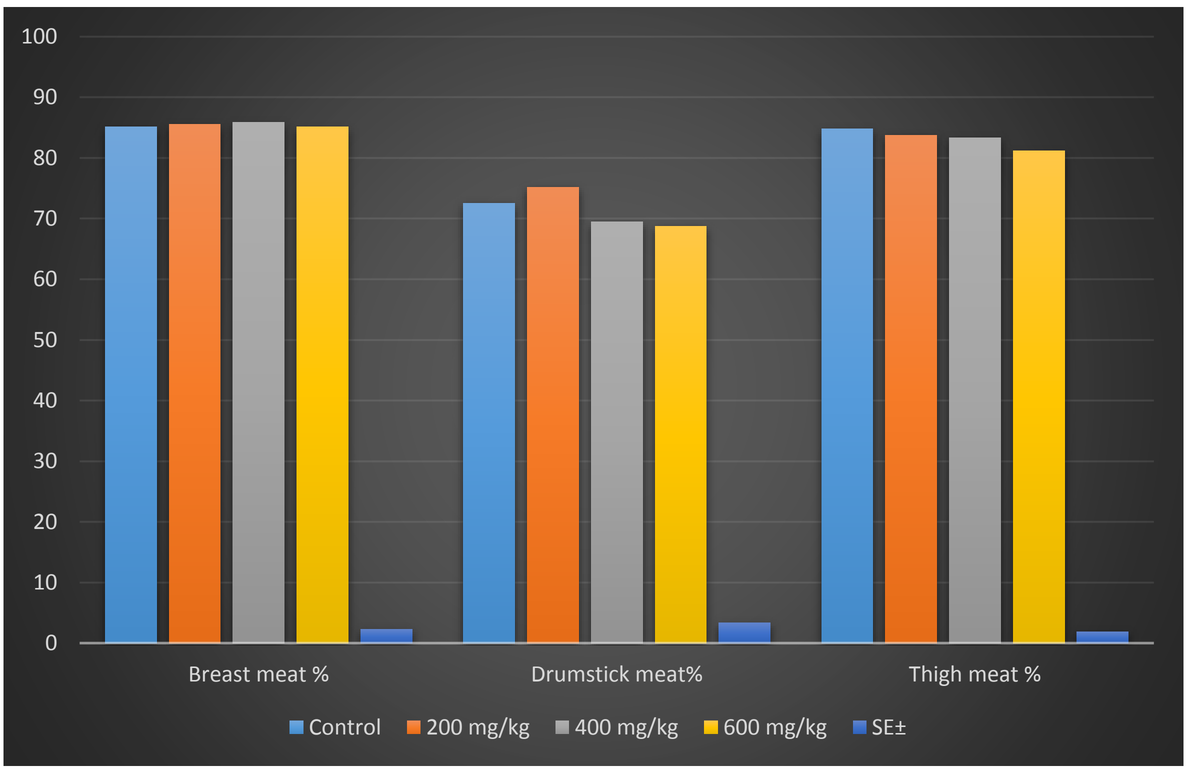


Fig.35: Effect of adding graded levels of Eucalyptus, Clove and Basil mixed essential oils on commercial cuts meat percentages

The results of sensory evaluation of chicks' meat fed on graded levels of Eucalyptus, Clove and Basil mixed essential oils were illustrated in table (42) and figure (36).

For juiciness there were no significant ($P \geq 0.05$) difference between group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 400 mg/kg mixed oils, compared with group of chicks fed on 600 mg/kg mixed oils and group of chicks fed on control diet which had the highest level, and for color no significant ($P \geq 0.05$) difference between group of chicks fed on control diet and group of chicks fed on 200 mg/kg mixed oils, compared with group of chicks fed on 600 mg/kg mixed oils and group of chicks fed on 400 mg/kg mixed oils which had the lowest, and for flavor no significant ($P \geq 0.05$) difference between group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils, compared with group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on control diet which had the lowest, also for tenderness, no significant ($P \geq 0.05$) difference between group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 400 mg/kg mixed oils, compared with group of chicks fed on 600 mg/kg mixed oils and group of chicks fed on control diet which had the highest.

Table (42): Sensory evaluation of chick's meat fed on graded levels of Eucalyptus, Clove and Basil mixed essential oils:

Treatments	Juiciness	Color	Flavor	Tenderness
Control	6.130^{a}	6.060^{a}	5.450^{b}	6.110^{a}
200 mg/kg	5.420^{b}	5.960^{a}	5.610^{a}	5.690^{c}
400 mg/kg	5.440^{b}	5.570^{c}	5.560^{ab}	5.660^{c}
600 mg/kg	5.500^{ab}	5.780^{b}	5.600^{a}	5.860^{b}
SE±	0.205	0.036	0.047	0.042

Values are mean± SD, Any two mean value(s) bearing different superscript(s) in a column are significantly different (P≥0.05) according to DMRT, SE ± Standard Error

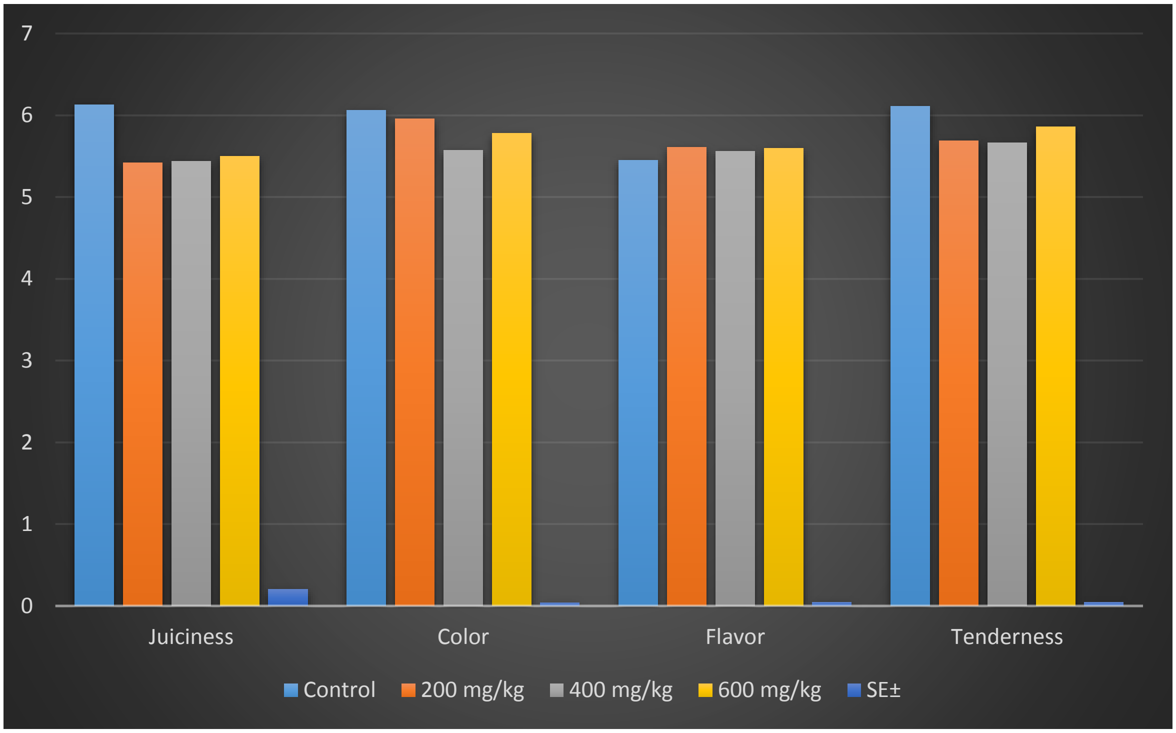


Fig. 36: Sensory evaluation of chick's meat fed on graded levels of Eucalyptus, Clove and Basil mixed essential oils

The results of adding graded levels of Eucalyptus, Clove and Basil mixed essential oils on meat chemical composition were showed in table (43) and figure (37).

For moisture no significant ($P \geq 0.05$) difference between group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils, compared with group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on control diet which had the lowest, also for dry matter no significant ($P \geq 0.05$) difference between group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils, compared with group of chicks fed on control diet which had the highest and group of chicks fed on 200 mg/kg mixed oils which had the lowest, while for ash no significant ($P \geq 0.05$) differences among all tested groups, but for crude protein there were significant ($P \leq 0.05$) differences among all tested groups, group of chicks fed on 200 mg/kg mixed oils had the highest, and group of chicks fed on 600 mg/kg mixed oils had the lowest, compared with group of chicks fed on control diet and group of chicks fed on 400 mg/kg mixed oils, and for ether extract no significant ($P \geq 0.05$) differences were observed between group of chicks fed on control diet, group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils, compared with group of chicks fed on 200 mg/kg mixed oils which had the lowest.

Table (43): Effect of adding graded levels of Eucalyptus, Clove and Basil mixed essential oils on meat chemical composition:

Treatments	Moisture%	Dry-matter%	Ash %	Crude protein%	Ether extract%
Control	73.233^{b}	26.767^{a}	1.200^{a}	22.583^{c}	1.377^{a}
200 mg/kg	75.533^{a}	24.467^{b}	1.300^{a}	28.973^{a}	0.400^{b}
400 mg/kg	74.533^{ab}	25.467^{ab}	1.200^{a}	23.437^{b}	1.300^{a}
600 mg/kg	74.900^{ab}	25.100^{ab}	1.250^{a}	21.810^{d}	1.150^{a}
SE±	0.644	0.644	0.050	0.171	0.157

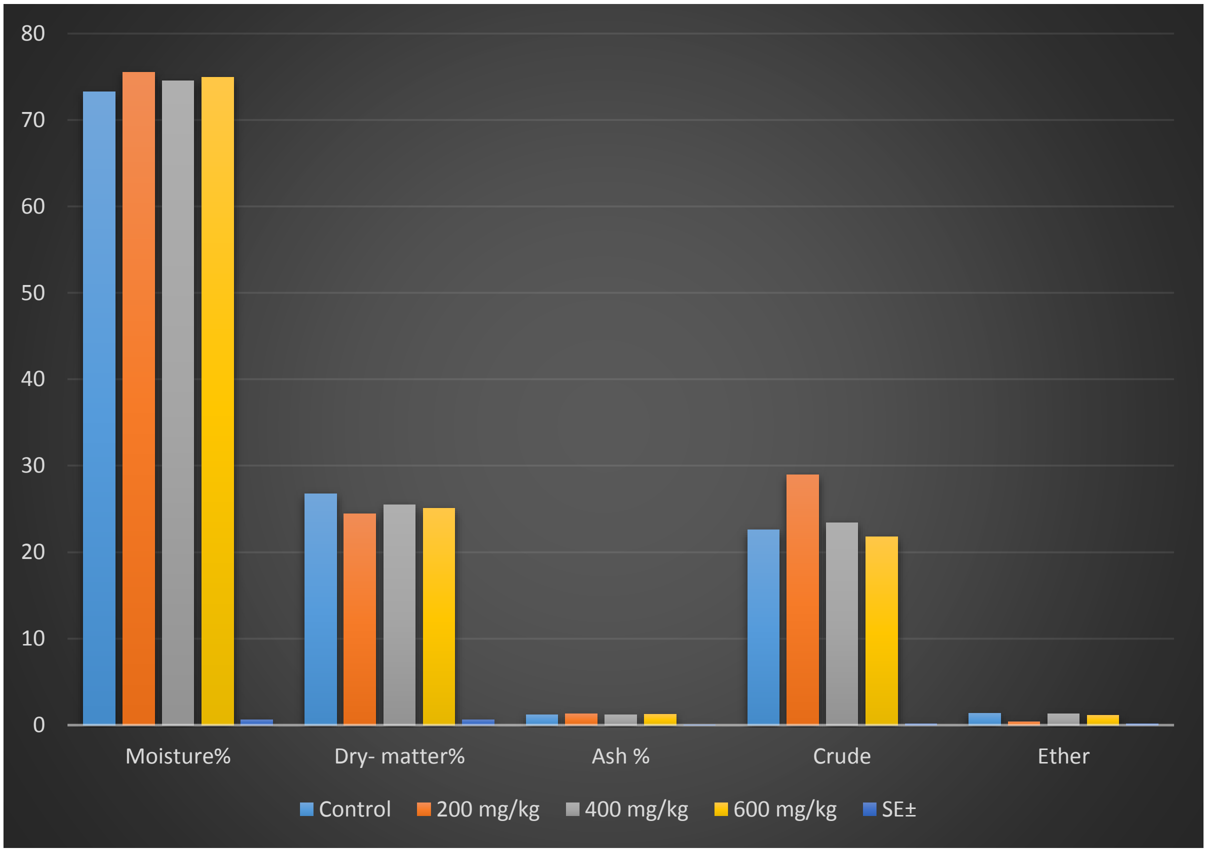


Fig. 37: Effect of adding graded levels of Eucalyptus, Clove and Basil mixed essential oils on meat chemical composition

The results of adding graded levels of Eucalyptus, Clove and Basil mixed essential oils on blood serum metabolites were illustrated in table (44) and figure (38).

For glucose there were significant ($P \leq 0.05$) differences between all tested groups, group of chicks fed on 600 mg/kg mixed oils had the highest level, then group of chicks fed on 400 mg/kg mixed oils then group of chicks fed on 200 mg/kg mixed oils while group of chicks fed on control diet had the lowest, for triglyceride, no significant ($P \geq 0.05$) difference between group of chicks fed 200 mg/kg mixed oils and group of chicks fed on 400 mg/kg mixed oils, compared with group of chicks fed on control diet and group of chicks fed on 600 mg/kg mixed oils which had the highest level, also for total protein no significant ($P \geq 0.05$) difference between group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils, compared with group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on control diet which had the highest, and for albumin there were no significant ($P \geq 0.05$) difference between group of chicks fed on control diet and group of chicks fed 200 mg/kg mixed oils, compared with group of chicks fed on 600 mg/kg mixed oils and group of chicks fed on 400 mg/kg mixed oils which had the lowest, for cholesterol there were significant ($P \leq 0.05$) differences among all tested groups, group of chicks fed on 200 mg/kg mixed oils had the highest level, then group of chicks fed on 600 mg/kg mixed oils, then group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on control diet had the lowest level, for HDL there were significant ($P \leq 0.05$) differences between all tested groups, group of chicks fed on 200 mg/kg mixed oils had the highest level, then group of chicks fed on 600 mg/kg mixed oils, then group of chicks fed on control diet while group of chicks fed on 400 mg/kg mixed oils had the lowest level, also for LDL there were significant ($P \leq 0.05$) differences between all tested groups, group of chicks fed on 200 mg/kg mixed oils had the highest level, then group of chicks fed on 600 mg/kg mixed oils, then group of chicks fed on 400 mg/kg mixed oils, and group of chicks fed on control diet had the lowest, for urea no significant ($P \geq 0.05$) differences between group of chicks fed on 200 mg/kg mixed oils, group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils, compared with group of chicks fed on control diet which had the highest, also for uric acid there were significant ($P \leq 0.05$) differences among all tested groups, group of chicks fed on 200 mg/kg mixed oils had the highest level, then group of chicks fed on 400 mg/kg mixed oils, then group of chicks fed on 600 mg/kg mixed oils, while group of chicks fed on control diet had the lowest, for creatinine no significant ($P \geq 0.05$) differences between group of chicks fed on 200 mg/kg mixed oils, group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils, compared with group of chicks fed on control diet which had the lowest.

Table (44): Effect of adding graded levels of Eucalyptus, Clove and Basil mixed essential oils on blood serum metabolites:

Treatments	Glucose (mg\dl)	Tri-gly Ceride (mg\dl)	Protein (g/dl)	Alb. (g/dl)	Cholest. (mg/dl)	Chol. HDL (mg/dl)	Chol. LDL (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	Creatine (mg/dl)
Control	220.50^{c}	43.500^{b}	3.950^{a}	2.050^{a}	124.50^{d}	$130.\ 50^{a}$	22.500^{d}	7.100^{a}	3.467^{d}	2.110^{c}
200 mg/kg	223.50^{c}	39.500^{c}	2.550^{c}	1.900^{ab}	143.50^{a}	137.300^{b}	38.500^{a}	4.500^{b}	6.667^{a}	2.440^{a}
400 mg/kg	229.00^{b}	40.500^{c}	2.950^{b}	1.600^{c}	130.00^{c}	129.500^{d}	30.500^{c}	4.000^{bc}	5.400^{b}	2.300^{b}
600 mg/kg	233.00^{a}	50.500^{a}	2.700^{c}	1.800^{b}	141.00^{b}	131.700^{c}	33.200^{b}	3.500^{c}	4.600^{c}	2.420^{ab}
SE±	1.118	0.408	0.054	0.061	0.646	0.289	0.363	0.292	0.133	0.043

Values are mean± SD, Means value(s) bearing no different superscript(s) in a column are not significantly different

($P \geq 0.05$) according to DMRT, SE ± Standard Error

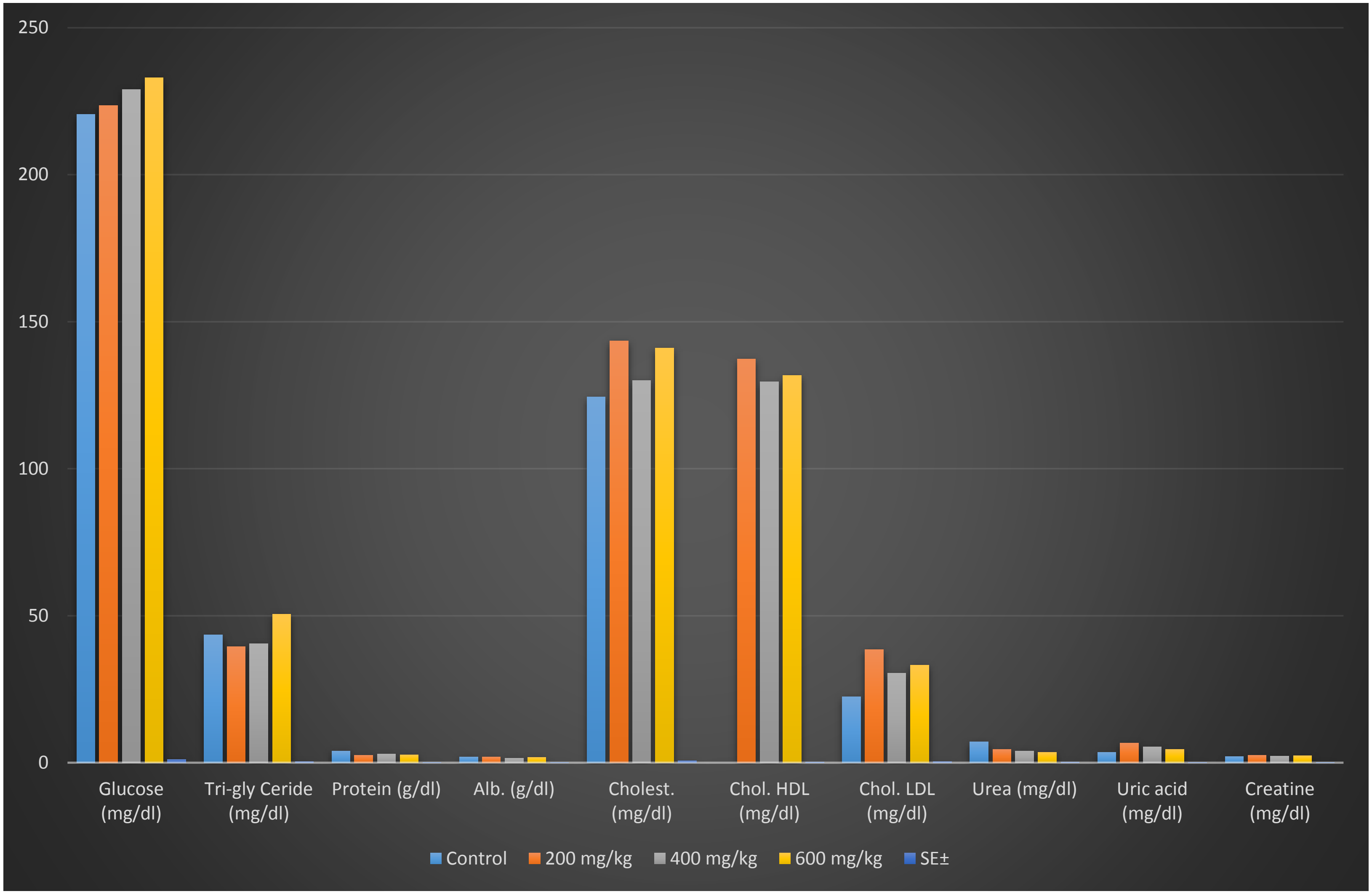


Fig.38: Effect of adding graded levels of Eucalyptus, Clove and Basil mixed essential oils on blood serum metabolites

The results of adding graded levels of Eucalyptus, Clove and Basil mixed essential oils on serum enzymes and minerals were shown in table (45) and figure (39).

For Aspartate Amino Transferase (AST) enzyme no significant ($P \geq 0.05$) differences between group of chicks fed on control diet, group of chicks fed on 200 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils, compared with group of chicks fed on 400 mg/kg mixed oils which had the lowest level, but for Alkaline Phosphatase (ALP) there were significant ($P \leq 0.05$) differences among all tested groups, group of chicks fed on control diet had the highest level, then group of chicks fed on 600 mg/kg mixed oils, then group of chicks fed on 400 mg/kg mixed oils, while group of chicks fed on 200 mg/kg mixed oils had the lowest.

For Ca ratio there were no significant ($P \geq 0.05$) difference between group of chicks fed on control diet and group of chicks fed on 200 mg/kg mixed oils, compared with group of chicks fed on 600 mg/kg mixed oils and group of chicks fed on 400 mg/kg mixed oils which had the lowest ratio, and for P there were significant ($P \leq 0.05$) differences among all tested groups, group of chicks fed on control diet had the highest, then group of chicks fed on 200 mg/kg mixed oils, then group of chicks fed on 600 mg/kg mixed oils and group of chicks fed on 400 mg/kg mixed oils.

Table (45): Effect of adding graded levels of Eucalyptus, Clove and Basil mixed essential oils on serum enzymes and minerals:

Treatments	AST (iu/l)	ALP (iu/l)	Ca (mg/dl)	Po (mg/dl)
Control	28.950[a]	247.50[a]	8.150[a]	8.750[a]
200 mg/kg	29.550[a]	165.25[d]	8.150[a]	7.150[b]
400 mg/kg	25.950[b]	183.00[c]	5.900[c]	5.650[d]
600 mg/kg	29.450[a]	197.10[b]	7.400[b]	5.950[c]
SE±	0.394	0.928	0.087	0.091

Values are mean± SD, Means value(s) bearing no different superscript(s) in a column are not significantly different (P≥0.05) according to DMRT, SE ± Standard Error

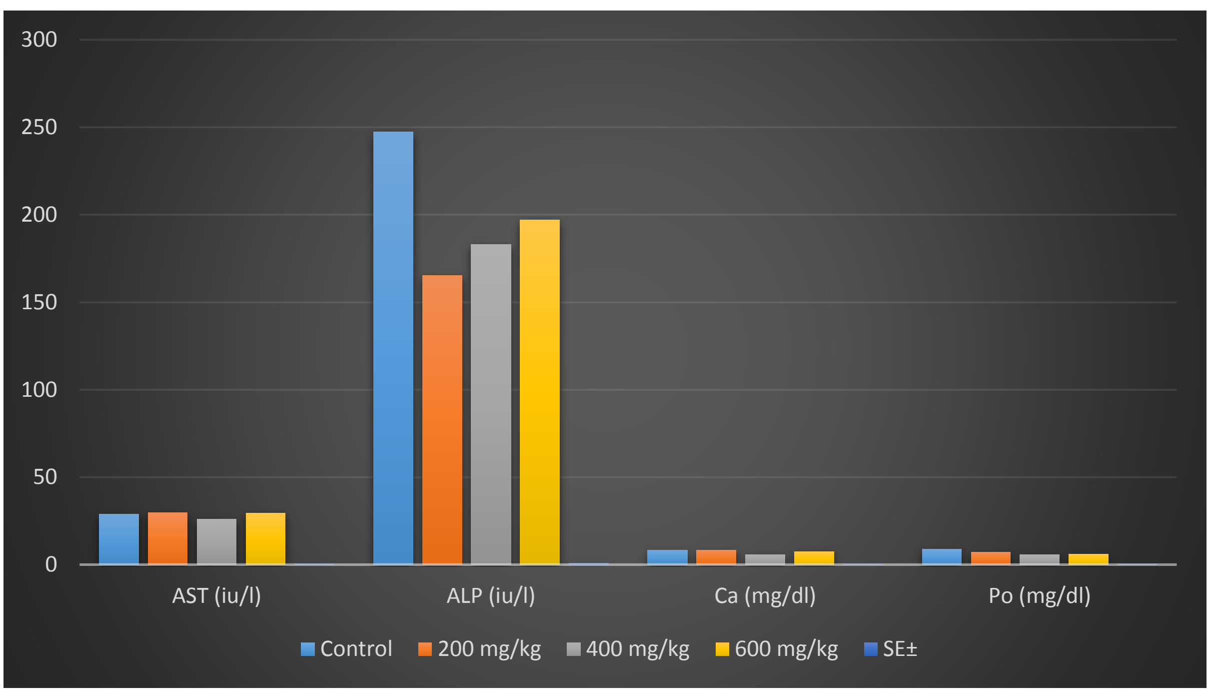


Fig.39: Effect of adding graded levels of Eucalyptus, Clove and Basil mixed essential oils on serum enzymes and minerals

Economical Appraisal:

Chicks purchase, feed cost, management, labor and electricity values were the main inputs considered. The total revenues obtained resemble the total selling values of the meat. Profitability ratio (1.202) for group of birds fed on 200 mg/kg mixed oils was the highest of the tested groups.

Table (46): Economical appraisal for broiler chicks fed on graded levels of Eucalyptus, Clove and Basil mixed essential oils:

Parameters	Control	200 mg/kg	400 mg/kg	600 mg/kg
Chick price	19.0	19.0	19.0	19.0
Diet cost	37.7	40.5	41.7	44.8
Labor	7.0	7.0	7.0	7.0
Total cost	63.7	66.5	67.7	70.8
Revenues	137.8	155.5	125.3	139.0
Profit	74.1	89.0	57.6	68.2
Profitability ratio	1.00	1.202	0.777	0.920

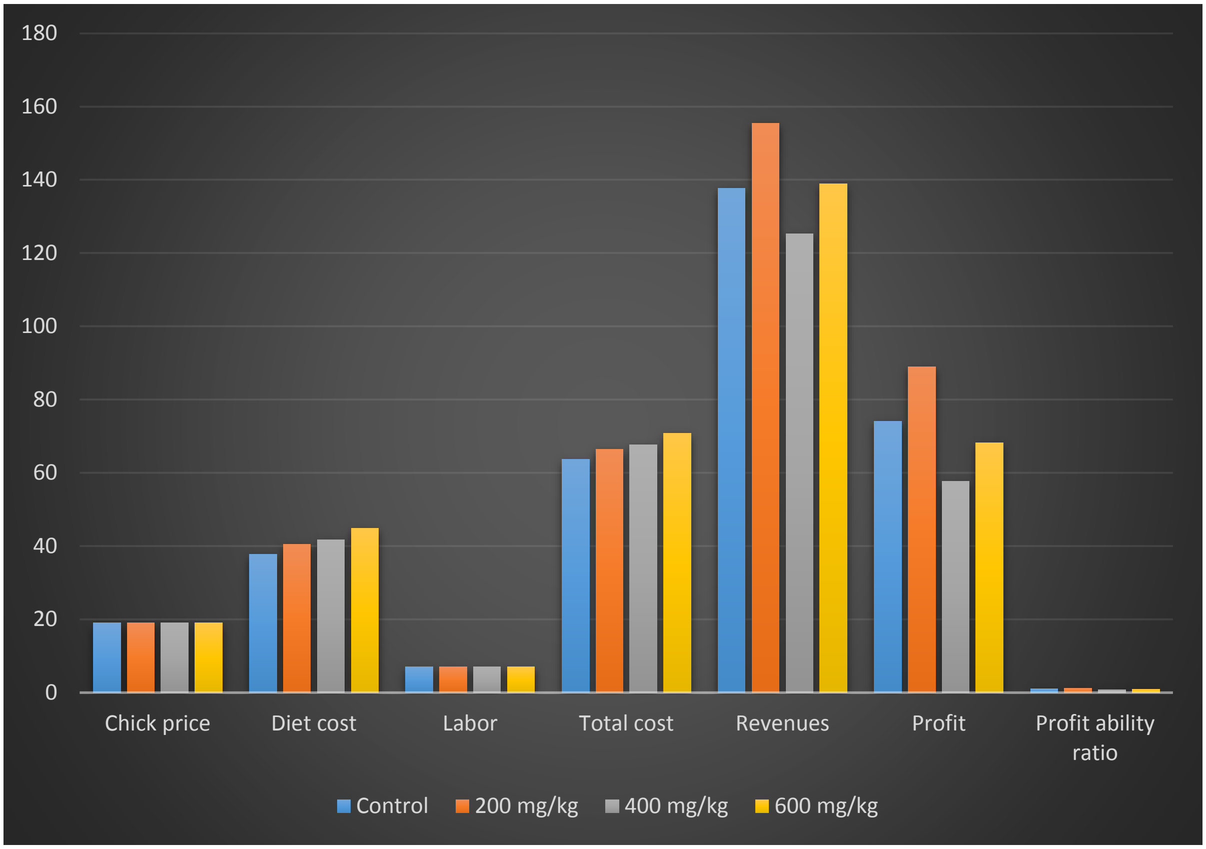


Fig.40: Economical appraisal for broiler chicks fed on graded levels of Eucalyptus, Clove and Basil mixed essential oils

The results of interactive impact of different blends and graded levels of essential oils on broiler chicks performance were shown in table (47) and figures (41, 42, and 43).

For body weight, there were no significant ($P \geq 0.05$) difference between experiment fed on clove and basil mixed oils and experiment fed on eucalyptus, clove and basil mixed oils, but experiment fed on eucalyptus and basil mixed oils had the highest body weight, whereas experiment fed on eucalyptus and clove mixed oils had the lowest, and for body weight gain there were no significant ($P \geq 0.05$) differences among all experiments groups, however experiment fed on eucalyptus and basil gained the highest, and experiment fed on eucalyptus and clove mixed oils had the lowest, also for feed intake no significant ($P \geq 0.05$) diffrernces among all experiments groups, however experiment fed on clove and basil mixed oils had the best, and for feed conversion ratio there were no significant ($P \geq 0.05$) difference between experiment fed on eucalyptus and basil mixed oils and experiment fed on eucalyptus, clove and basil mixed oils, but experiment fed on clove and basil mixed oils had the better. All these experiments results obtained were better than control one.

Results obtained for level effect declared that, for body weight there were no significant ($P \geq 0.05$) differences among all inclusions, however inclusion of 600 mg/kg mixed oils had the highest, and inclusion of 400 mg/kg had the lowest, also for body weight gain there were no significant ($P \geq 0.05$) differences among all inclusions, however inclusion of 200 mg/kg mixed oils had the highest, and inclusion of 600 mg/kg had the lowest, for feed intake and feed conversion ratio there were no significant ($P \geq 0.05$) differences among all inclusions, but inclusion of 400 mg/kg had the better.

Table (47): Interactive Impact of Different Blends and Graded Levels of Essential Oils on Broiler Chicks Performance:

Treatments		BW	BWG	FI	FCR
CONTROL		1,942.00	1,757.00	3,428.00	1.95
EXPERIMENT EFFECT					
1	EXP1	1,999.56 [b]	1,814.67	3,425.22	1.90[a]
2	EXP2	2,239.22 [a]	1,943.11	3,421.67	1.77[ab]
3	EXP3	2,114.22 [ab]	1,929.22	3,354.67	1.74[b]
4	EXP4	2,033.33 [ab]	1,848.33	3,393.00	1.84[ab]
	SEM	72.3797	59.92	58.80	0.052
	p -value	0.1181	0.3769	0.8189	0.1546
	Sig		N.S	N.S	
LEVEL EFFECT					
1	200 g/Kg	2,103.75	1,918.75	3,463.50	1.81
2	400 g/Kg	2,067.83	1,882.83	3,325.58	1.78
3	600 g/Kg	2,118.17	1,849.92	3,406.83	1.86
	SEM	62.6826	51.89	50.93	0.46
	p -value	0.8438	0.6491	0.7185	0.4502
	Sig	N.S	N.S	N.S	N.S
EXP X LEVEL INTERACTION					
	SEM	125.365	103.79	101.85	0.09
	p -value	0.4742	0.8734	0.7871	0.7656
	Sig	N.S	N.S	N.S	N.S

EXP1: Eucalyptus and Clove

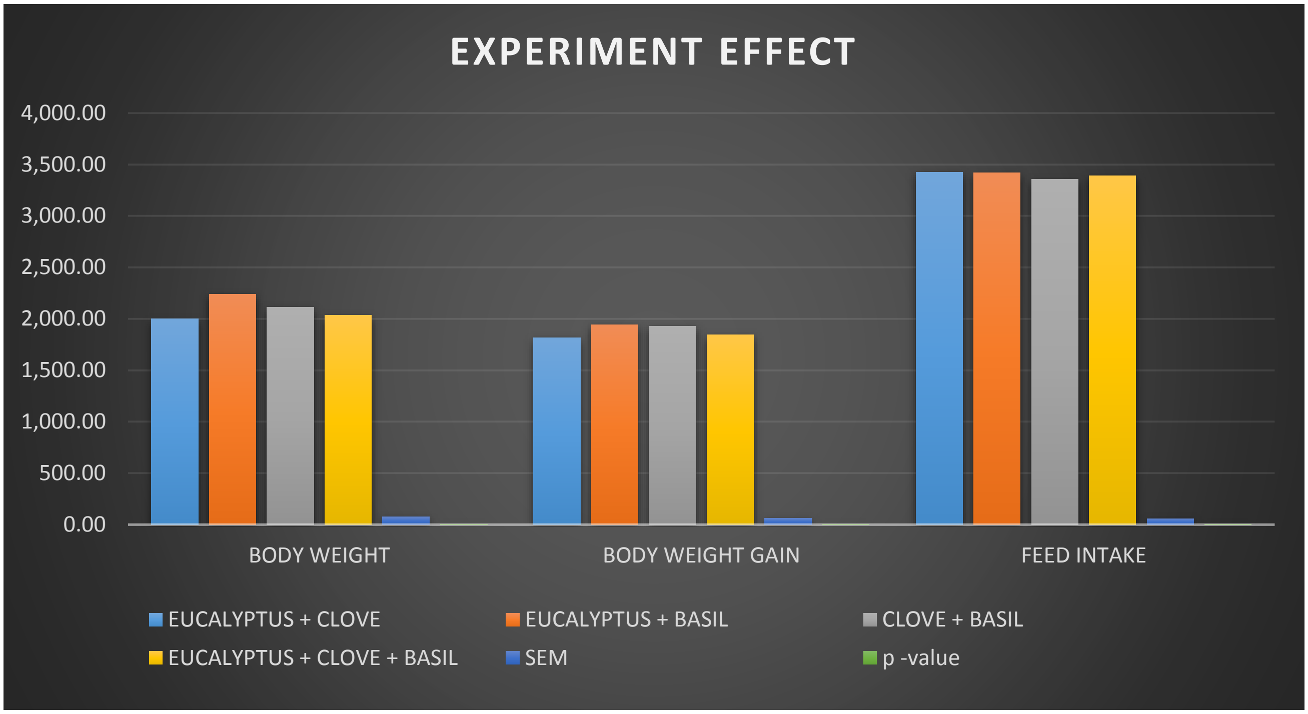
EXP2: Eucalyptus and Basil

EXP3: Clove and Basil

EXP4: Eucalyptus, Clove and Basil

N.S: No Significant

SEM: Standard Error of the Mean



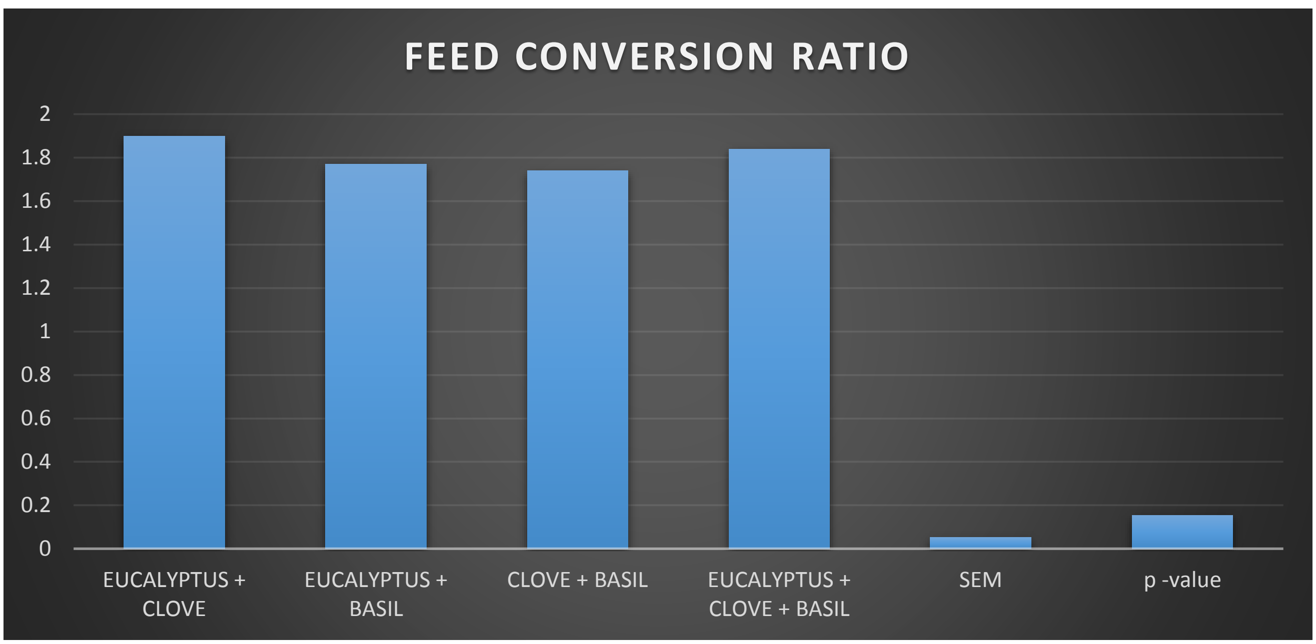
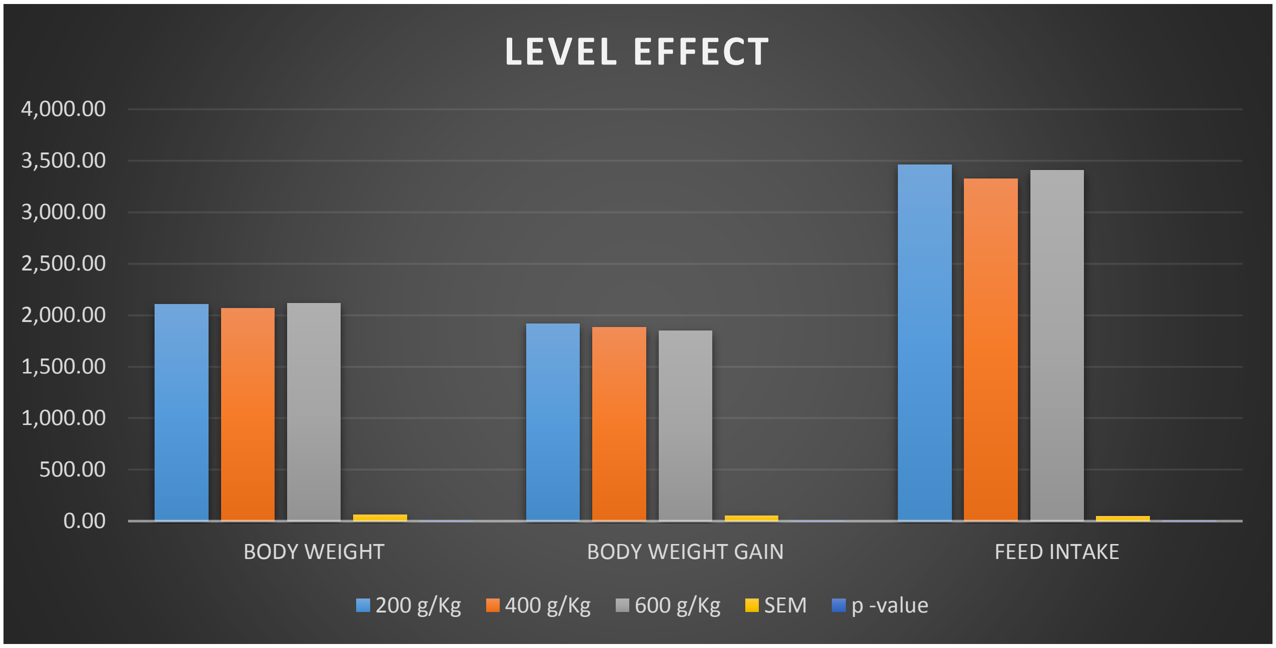


Fig.41: Interactive Impact of Different Blends and Graded Levels of Added Essential Oils on Broiler chicks Performance



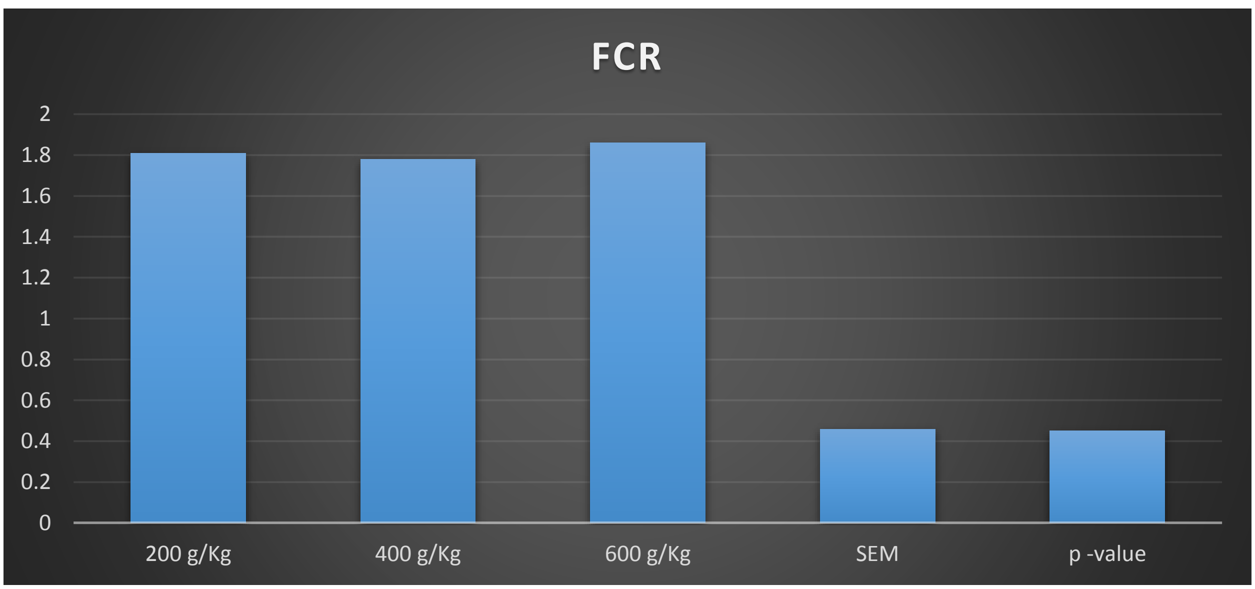
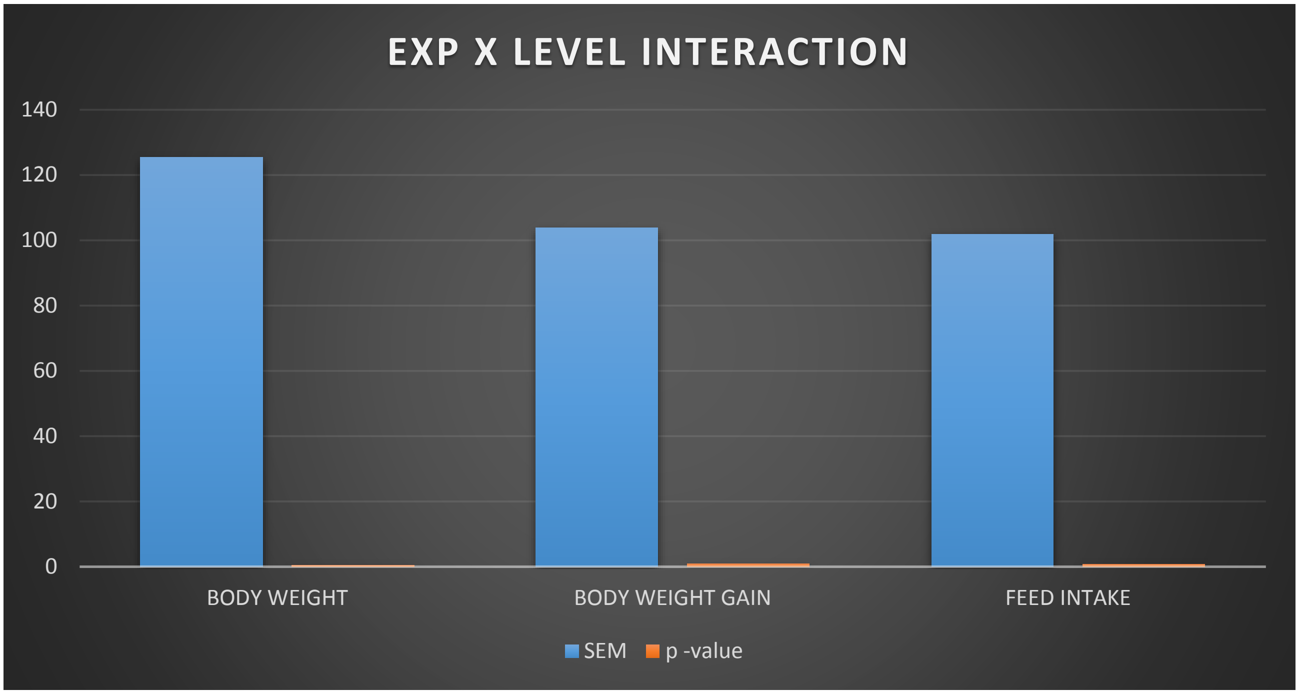


Fig.42: Interactive Impact of Different Blends and Graded Levels of Added Essential Oils on Broiler chicks Performance



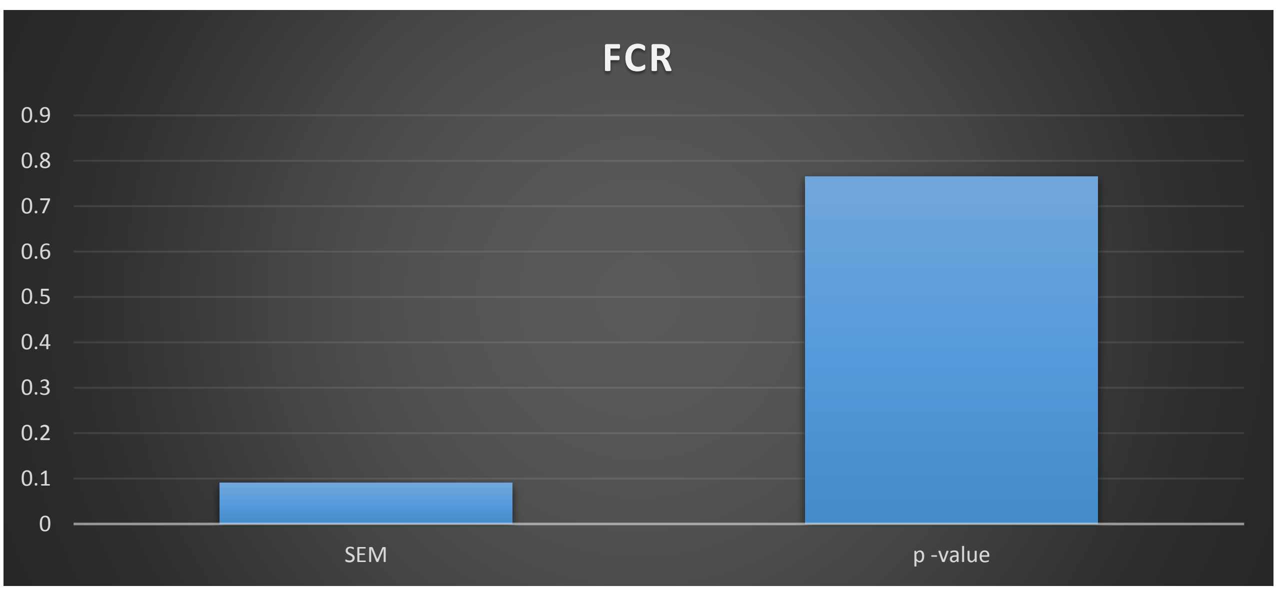


Fig.43: Interactive Impact of Different Blends and Graded Levels of Added Essential Oils on Broiler chicks Performance

CHAPTER FIVE
DISSCUSION

5. 1 Experiment (1):

Response of broiler chicks fed on graded levels of Eucalyptus and Clove mixed essential oils (ECMEO):

Most essential oils consist of mixture of compounds such as phenolics of polyphenols, terpenoids, sapcnines, quinme, esters, flavcne, flavonoids, tannins, alkaloids and non-volatile residues and their chemical composition of concentration of compounds is variable.

Chemical analysis of eucalyptus essential oil in our study reported that, (D-(+)-Camphor, Cis-2-Menthenol, Elemol, Cedrenol, Gurjunene and Cis-Lanceol) were the main compounds. These results were in line to some extent with the results obtained by (Akin *et al.,* 2010; Chalchat *et al.,* 2001), they found that 1.8- Cineole, a- phellandrebe, Elemol, Cedrenol, p- Cymene and other constituents. But (Khaled *et al.*, 2015) identified monoterpene hydrocarbons as the major constituents. whereas results were in contrast to those finding by; (Bugarin *et al.,* 2014), who said 1,8 Cineole was confirmed as the main component of eucalyptus essential oil, also (Issam *et al.,* 2018) reported that, the main compounds of chemical analysis of eucalyptus leaf essential oil were, phellandrene, dimethyline, trimethylene.

The chemical composition of the essential oil defines its mode of action as well as its attributes. Differences between or within essential oils depend significantly on several variables, such as plant species, physical and chemical soil conditions, harvest time, degree of plant maturity, technology of drying, duration of storage and extraction process (Burt., 2004; Bakkali *et al.,* 2008).

Data about the chemical composition of the essential oil taken from different literature also suffers from this indisposition. This can also be due to lacks of standardisation implying differences in analytical methods such as gas chromatography or mass spectrometry. Varying results lead to difficulties in using essential oils, since optimal dosage and mixture are yet to be identified. Thus, it is meaningful to define active chemical components in essential oil of some selected herbs, which are commonly used as feed additives in broiler diets.

There are few reports regarding the effect of eucalyptus and clove mixed essential oils in poultry, in our study the apparent health of experimental stock was good through-out the experimental period. The general behavior of the stock also was good. The ambient temperature during the experimental period fell within the thermo neutral zone has extracted no heat on the experimental birds, no mortalities were recorded among the different treatment groups through-out the experimental period and this may be due to the good hygiene conditions, and it could be suggested that the actives substances contained in the mixed essential oils product, might have had a positive effect on gut micro flora by reducing the amount of pathogenic bacteria. Similar results were obtained by (Mukhtar., 2011; Mukhtar *et al.*, 2013), who reported that essential oils as natural feed additive in broiler diets decreases the mortality rate due to its active ingredients which are acts as anti-microbial agents.

The performance results of broilers fed on diets supplemented with graded levels of Eucalyptus and Clove mixed essential oils (Table 5), showed no significant ($P \geq 0.05$) differences between birds fed on control diet and group fed on 200 mg/kg, 400 mg/kg and 600 mg/kg mixed essential oils, in final body weight, although group of birds fed on 200 mg/kg mixed oils had the heaviest weight than groups fed on 400 mg/kg and 600 mg/kg mixed essential oils, which were showed the lowest weight. The reason of reducing in 400 mg/kg and 600 mg/kg mixed essential oils, might be due to high essential oil concentration, mixed essential oils group could be affected negatively digestive system. Besides, essential oils positively affected digestibility of nutrients. These results are in accordance with the reports of Farhadi *et al.*, (2017), who found that dietary addition of eucalyptus essential oil (EEO) had no effect on body weight gain and feed conversion ratio during different growth periods compared with the control. In contrast, Issam *et al.,* (2018), found that feeding broiler chicks on different levels of essential eucalyptus oil (ECO) recorded positive significant differences in live body weight and body weight gain. However, group fed on diet supplemented with 400 g/kg (ECO) recorded significantly the heaviest weight compared with the other tested groups. This might be due to the therapeutic properties of the essential eucalyptus oil Khaled *et al.,* (2015) and or its bioactive products that showed anti-bacterial, anti-fungal Su *et al.,* (2006), anti- inflammatory effects and anti-oxidant activities (Siramon and Ohtani, 2007).

Several studies confirmed the positive influence of herbs and their essential oils on body weight gain by their ability to destroy pathogen micro-organisms in the digestive system and consequently increasing the production of digestive enzymes which improve utilisation of digestive products (Toghyani *et al.,* 2010). Although, in this study, results obtained showed no significant differences between all tested groups in body weight gain through-out the experimental period, however group of chicks fed on 200 mg/kg mixed essential oils had numerically the highest body weight gain compared with other tested groups during the experimental period. Result was similar to some extend with Daffallaa and Mukhtar., (2016), who revealed no significant difference in body weight gain and feed conversion ratio, although group fed on 200 g/kg mixed essential oils recorded the best values, this might be due to more feed intake compared with the control group, also this statement could be in line with founding's of Mukhater *et al.,* (2013), who observed no effects on growth performance with essential oils supplementation. In contrast, Mohamed and Mukhtar., (2017) showed significantly improved in final body weight and body weight gain of the chicks fed on diet supplemented with different levels essential oils mixture derived from caraway and anise.

Feed intake which impacts body weight gain with different efficiency will determine the final body weight. The results in this study showed that application of Eucalyptus and Clove mixed essential oils, observed no significant differences between all tested groups in feed intake. However group of chicks fed on 200 mg/kg and 600 mg/kg mixed essential oils consumed more feed than those group of chicks fed on control diet while feed intake for group fed on 400 mg/kg mixed essential oils decreased during the experimental period, this might be due to that essential oils (EOs) improved diet palatability, enhancing appetite of chicks and or quicker digestion and passage of nutrients through the digestive effects of essential oils, and can stimulate animal digestive system. These results were consistent with the findings of Mukhater *et al.,* (2013), Daffallaa and Mukhtar, (2016), when they added mixture of three herbal essential oils (Anise essential oil, Caraway essential oil and Clove essential oil). however, the results of this study contradict with Issam *et al.,* (2018), who found that feeding broiler chicks on diets supplemented with mixed essential oils (MEO) from eucalyptus essential oil (EEO), fenugreek essential oil (FEO) and fennel essential oil (FEO), showed positive significant effects on broiler chicks performance, on the same trend, Erener *et al.,* (2010), and Ismail *et al.,* (2011) recorded more feed consumption when added black cumin oil. However, Borazjanizadeh *et al.,* (2011), reported decreased in feed intake with the increasing level of eugenol of clove leaf essential oil in broiler feed, caused by the lower feed palatability. Also, Abaza *et al.*, (2008), who found that the addition of black cumin oil in the diet reduced significantly feed consumption of broiler chicks.

Feeding broilers on diets supplemented with graded levels of Eucalyptus and Clove mixed essential oils revealed no significant differences between all tested groups in feed conversion ratio, but group of chicks fed on 200 mg/kg mixed oils recorded the best feed conversion ratio compared with the control group and group of chicks fed on 400 mg/kg and 600 mg/kg mixed essential oils. These results were agreed with that obtained by Daffallaa and Mukhtar, (2016), who found no significant differences in body weight gain and feed conversion ratio, and group fed on 200 g/kg mixed essential oils recorded the best values. And this might be due to more feed intake compared with the other tested groups.

As overall, the performance results of broilers fed on diets supplemented with graded levels of Eucalyptus and Clove mixed essential oils showed no significant differences between chicks fed on control diet and groups fed on 200 mg/kg, 400 mg/kg and 600 mg/kg mixed essential oils, in final body weight, although group of chicks fed on 200 mg/kg mixed oils had the heaviest weight than groups of chicks fed on 400 mg/kg and 600 mg/kg mixed essential oils. These results were contradict with Issam *et al* ., (2018), who found that feeding broiler chicks on diets supplemented with mixed essential oils (MEO) from eucalyptus essential oil (EEO), fenugreek essential oil (FEO) and fennel essential oil (FEO), obtained positive significant effects on the broiler chicks performance, (body weight, feed intake and body weight gain).

Results showed no significant differences among all tested groups in dressing and giblets percentages (Table 6), non-carcass (Table 7), commercial cuts

(Table 8) and their separable tissue percentages (Table 9), meat chemical composition (Table11) which was confirmed with the panel taste values (Table 10). The results were in agreement with founding's of (Erener *et al.*, 2010; Ismail *et al.*, 2011; Mukhtar *et al.*, 2011; Daffallaa and Mukhtar, 2016; Issam *et al.*, 2018). But in contrast with Hamodi and Khalani, (2011). Also Mohamed and Mukhtar, (2017), recorded a significant increase in carcass cuts, the percentage of abdominal fat, giblets (liver, heart and gizzard) with the inclusion of mixed essential oils compared with the control group.

The blood serum metabolites analysis (Table 12) showed, no significant differences between groups fed diets supplemented with mixed essential oils for parameters of glucose, triglyceride, total protein, albumin, cholesterol HDL, cholesterol LDL, urea and creatinine compared with control group. Whereas chicks fed on control diet recorded the highest concentration for triglyceride, total protein, cholesterol HDL and serum urea, while chicks fed on diet supplemented with 600 mg/kg mixed oils had the lowest values for triglyceride, total protein, albumin and creatinine. This might be due to the oil active ingredients which inhibit hepatic 3-fenchyc-3-methyl glutary co-enzyme A (HMG-CO A reductase activity which is a key regulatory enzyme in cholesterol synthesis, and thymoquinone which could have stimulated the excretion of uric in urine. The result was in line to the finding of (Rahimi *et al.*, 2011; Mukhtar *et al.*, 2013). But for uric acid there was significant differences among all tested groups, group of chicks fed on 200 mg/kg mixed oils had the highest level and group of chicks fed on control diet had the lowest. This results were equally in harmony with the findings of (Issam *et al.*, 2018). Protein and cholesterol with the increase of essential oils mixed levels in the diet. However, diet supplemented by 200 mg/kg mixed essential oils showed the lowest concentration for above mentioned parameters.

The results of adding graded levels of Eucalyptus and Clove mixed essential oils on serum enzymes and minerals (Table 13) showed no significant ($P \geq 0.05$) differences for AST and ALP enzymes between groups of chicks fed on control diet, group of chicks fed on 400 mg/kg and group of chicks fed on 600 mg/kg mixed oils compared with group of chicks fed on 200 mg/kg mixed oils which recorded significantly the lowest values. The result was in agreed with the findings of (Dieumon, 2009; Mukhtar *et al.*, 2013).

Results obtained recorded significant ($P \leq 0.05$) differences for Ca levels in blood serum between dietary treated groups compared with control group, also for P level there were significant differences between group of chicks fed on 400 mg/kg mixed oils and group of chicks fed on 600 mg/kg mixed oils compared with group of chicks fed on 200 mg/kg mixed oils and control group. These differences among the groups in parameters might be due to active ingredient. Results were in line with Issam *et al.,* (2018), who found that there were significant differences for Ca levels between all tested groups.

Result of economical evaluation (Table 14) showed that, group of chicks fed on 200 mg/kg mixed oils had Profitability ratio (1,149) which was the highest ratio compared with control group. So the addition of dietary mixed essential oils improved the performance of broiler chicks and resulted in economic benefits, and this in harmony with result obtained by (Issam *et al.,* 2018).

5. 2 Experiment (2):

Rresponse of broiler chicks fed on graded levels of Eucalyptus and Basil mixed essential oils (EBMEO):

The chemical analysis of Basil essential oil in our study declared, Eugenol methyl ether, Linalool, Methyl iso eugenol, Eucalyptol, Anethole, a-Guaiene were the major Compounds, and these results were in line to somehow with the numerous in vitro studies reported that major components of basil essential oils including methyl chavicol, linalool, alpha-pinene, methyl eugenol, eugenol, (Prabuseenivasan *et al.,* 2006; Sienkiewicz *et al.*, 2013; Hanif *et al.*, 2011).

Results obtained for broiler chicks fed on diets supplemented with graded levels of eucalyptus and basil mixed essential oils, declared no significant effects on growth performance of the chicks (Table 16), The in-significant effects of the addition of (EBMEO) to the basal diet might be due to the fact that, the diets were iso-caloric and it is expected that the feed consumption could be similar also might be due to the similar environmental during this period, In addition to anti-microbial, anti-fungal, anti-oxidant and anti-inflammatory effects of (EBMEO). The three levels of infusion used in this study have shown increased body weight gain compared with control group. Murray *et al*. (1999) reported that the improvement in body weight might be due to the presence of fat soluble, and for unidentified factors and essential fatty acids in medicinal and aromatic plant. The findings of this research is supported by the results of Nweze and Ekwe., (2012), who reported no significant difference in the performance of broilers fed on diet supplemented with basil extract. Also the results in harmony with the finding of Ulupi *et al.*, (2015), who found that the addition of different levels of basil flour in chicks feed did not affect on their performance, however group of chicks fed on 600 mg/kg mixed oils showed high body weight and this might be due to more feed intake to this group, on the other hands chicks fed on 200 mg/kg mixed oils had the highest body weight gain and better feed conversion ratio. There was no significant difference in feed conversion ratio showed between chicks fed on diets supplemented with graded levels of (EBMEO), and control group. While chicks fed the diets supplemented with (EBMEO) at graded levels had the best feed conversion ratio value as compared with control group. These results agreed with the finding of El-Gendi *et al.*, (1994), who indicated that there was an improvement in feed conversion ratio with feeding herbal products as feed additives that could be attributed to their effect on improving the digestibility of dietary protein in the small intestine.

These finding were disagreed with those of Azouz, (2001), who noted that adding fenugreek to broiler diet resulted in increased body weight. Also results were in contrast to that obtained by Abbas *et al.*, (2010); Osman *et al.*, (2010); Onwurah *et al.,* (2011), whom reported that addition of basil leaf and seed to the diet had a beneficial effect on feed intake, body weight gain and feed conversion ratio. Those results disagreed with Rabia, (2010), who reported that chicks fed basil diets had significantly heaviest body weights than those fed the control and fenugreek diets.

In the present study, results agreed with the finding of Nweze and Ekwe, (2012), who found no difference in the performance of broilers when basil extract was supplemented in diets. Also the results in harmony with the finding of Ulupi *et al.*, (2015), who showed that the addition of 1, 2 and 3% basil flour in chick feed did not have an effect on feed consumption, body weight and feed conversion ratio.

Also results observed no significant effects on the broiler dressing and giblets (liver, heart and gizzard) (Table 17), non-carcass components (Table 18), commercial cuts and their meat values (Table 19, 20), which was confirmed with the panel taste (Table 21). These results were in the same findings of Mukhtar., (2011); Amal *et al.*, (2013); Sadarman *et al.,* (2018), whom found that, the addition of the basil leaf meal in the commercial diets for broilers up to 9% did not significantly increase their weight and length of digestive organs of broilers to a maximal capacity. Findings of the present study also are supported by Abbas.,(2010); Gurbuz and Ismael., (2016), whom presented that feeding of 3 g/kg of fenugreek, parsley and basil seeds had not significantly affected liver, carcass and abdominal fat. But they were disagreed with the findings of Alcicek *et al.,* (2004), who found an improvement in dressing percentage by the supplementation with dietary essential oils.

Glucose results (Table 23) had no significant between control group and group fed on 200 mg/kg mixed oils and no significant between group fed on 400 mg/kg and group fed on 600 mg/kg mixed oils, Tri-glyceride results for chicks fed on 200 mg/kg and 400 mg/kg mixed oils registered significant low in concentrate of tri-glyceride level compared with control and 600 mg/kg mixed oils. For Cholesterol, there were no significant among groups. Urea, treated groups showed significant low concentrate compared with control. Uric acid and creatinine concentrate showed significant differences, although group fed on 400 mg/kg mixed oils recorded the highest concentrations. The decrease of cholesterol might be due to the active ingredients of the essential oils which inhibit hepatic 3-fenchyc-3-methyl glutary co-enzyme A (HMG-CO A reductase activity which is a key regulatory enzyme in cholesterol synthesis, but the reduction of urea level might be due to the active compounds such as thymoquinone which could have stimulated the excretion of uric in urine. These results could be supported by the findings of Rahimi *et al.,* (2011); Mukhtar *et al.,* (2013), when added different dietary levels of black cumin oil and spearmint oil as a natural growth promoters**.**

The effect of treatment was not significant on aspartate amino transfers (AST) enzyme (Table 24), except group fed on 600 mg/kg mixed oils which showed significantly high concentration. Alkaline phosphatase (ALP) enzyme, results revealed significant effects among all tested groups whereas control group showed significantly high concentration value. These results on enzyme activities could be supported by the findings of Dieumon *et al.*, (2009); Mukhtar *et al.,* (2013), whom found similar results when added different levels of black cumin oil and spearmint oil in different studies. On the other hand, our results in contrast with the finding of Kadhim *et al.*, (2016), who reported an improvement in biochemical parameters of the blood, especially total protein and a decrease in liver enzymes AST and ALP and cholesterol, when broilers fed on diets including 0.3% and 0.6% basil seeds.

Results obtained recorded no significant differences for calcium level in blood serum between treated groups, but for phosphorus, chicks fed on control diet showed high significant difference compared with other tested groups. Results were supported with findings of Amal *et al.,* (2013); Mukhtar *et al.,* (2013), when they used halfa bar essential oil (HBO) as a natural growth promoter in broiler nutrition.

There was no significant differences in commercial cuts percentages, their separable tissue, also chemical composition of meat was not affected significantly by dietary treatment, which was confirmed by the subjective meat quality values and results were in line with the findings of Mukhtar *et al.,* (2013) and Amal *et al.,* (2013), whom found similar results when they used halfa bar essential oil (HBO) as a natural growth promoter in broiler nutrition.

Result of economical evaluation (Table 25) showed that group of chicks fed on 400 mg/kg mixed oils recorded **(**1,147) as the highest profitability ratio compared with the control group. So the addition of dietary mixed essential oils improved the performance of broiler chicks and resulted in economic benefits.

5. 3 Experiment (3):

Response of broilers fed on graded levels of Clove and Basil mixed essential oils (CBMEO):

The chemical analysis of Clove essential oil in this study declared that Eugenol ,Trans-Isoeugenol, Isoeugenol, Chavicol, Kaempferol3,7,4-trimethyl ether,2',3',4', Trimethoxyacetophenone, were the major compounds, and these results were in line to somehow with the findings of Mohammadi *et al*., (2014), who found that the major components of clove essential oil are β–caryophyllene, eugenol and acethyleugenol. Whereas Ordoñez *et al*., (2008) found that the active ingredient of clove essential oil is eugenol.These variations in the main compounds might be due to the differentiation and the age of the plants.

Results of feeding broiler chicks on graded levels of Clove and Basil mixed essential oils (Table 27), were showed no significant ($P \geq 0.05$) effects in body weight, feed intake, body weight gain and feed conversion ratio among all tested groups, this in harmony with Mahrous *et al.*,(2017), who found no significant differences in growth performance (BWG and FCR) when fed broiler chickens on clove bud supplements at a rate of 0.5 and 1.0 g/kg diet, but performance deteriorated in groups supplemented with higher levels of cloves (1.5 g/kg diet). however group of chicks supplemented with 400 mg/kg mixed oils had the highest body weight, body weight gain and better feed conversion ratio compared with other tested groups, whereas group of chicks supplemented with 200 mg/kg mixed oils had more feed intake, and this in contrast with the finding of Mukhtar., (2011), who showed an increase in the feed intake by broiler chicks fed with 600 mg clove oil/kg as compared with those from the control and other treated groups, also results obtained were in contrary with the finding of Petrovic *et al.*,(2011), who said that supplementation of clove (Eugenia caryophyllus) in diet or drinking water of birds lead to impairment of their performance, and Agostini *et al*., (2012), reported that clove powder (0.1 - 2.5 g/kg diet) had a positive effect on growth performance, feed efficiency and changes in the intestine epithelium of broiler chickens. On the other hand many studies had reported that clove (Eugenia caryophyllus) was rich in trace minerals which essential for protein and carbohydrate metabolism. Reduced the synthesis of fatty acid and cholesterol that could be improved broiler performance (Hernandez *et al.*, 2004).

Also results declared no significant effects on the broiler dressing and giblets (liver, heart and gizzard) (Table 28), non-carcass components (Table 29), commercial cuts and their meat values which were confirmed with the panel taste (Table 30, 31, 32).

Glucose results for chicks supplemented with 400 and 600 mg/kg mixed oils registered (Table 34), significant low concentration level, which was agreed with the finding of Baker *et al*., (2008), who reported that uses of medicinal plants stimulating insulin secretion and prevent cellular resistance to insulin, reduced blood glucose levels. For tri-glyceride group supplemented with 200 mg/kg and 600 mg/kg mixed oils had no significant effects compared with group supplemented with 400 mg/kg mixed oils which had the highest level while control group had the lowest, this variation might be due to the concentration of the essential oils, protein result also registered no significant effects between groups supplemented with 400 mg/kg and 600 mg/kg mixed essential oils compared with group supplemented with 200 mg/kg mixed essential oils which had the lowest level and control group which had the highest level. Albumin level registered no significant effects among control group and groups supplemented with 200 mg/kg and 600 mg/kg mixed oils compared with group supplemented with 400 mg/kg essential mixed oils which had the lowest level, and these results were the same to some extend with the finding of Hassan and Awad., (2017), whom stated that 0.2%, 0.5%, and 0.8% dietary supplementation with thyme powder in broilers did not affect serum total protein and albumin when compared with that of the control group. Cholesterol level had no significant effects among control group; group supplemented with 400 mg/kg and 600 mg/kg mixed oils compared with group supplemented with 200 mg/kg mixed essential oils which had the lowest value, HDL results had no significant effects among control group, group supplemented with 200 mg/kg and 600 mg/kg compared with group supplemented with 400 mg/kg mixed essential oils which had the highest level, whereas LDL had significant effects among all tested groups. also urea level had no significant effects between group supplemented with 400 mg/kg and 600 mg/kg mixed oils compared with group supplemented with 200 mg/kg mixed oils and control group which had the highest level, but for uric acid no significant effects among all tested groups compared with control group, creatinine level registered no significant effects among control group, group supplemented with 200 mg/kg and group supplemented with 400 mg/kg mixed oils compared with group supplemented with 600 mg/kg mixed oils which had the highest level.

The effect of treatments had no significant effects on a aspartate amino transfers (AST) enzyme among all tested groups (Table 35), our finding in the same line with Mustafa., (2016), who showed no significant differences in AST, ALT, and ALP enzyme concentrations when broiler chickens were fed diet supplemented with mixed essential oils (clove, anise, and caraway) at a rate of 200, 400, and 600 mg/kg feed.

But there were significant ($P\leq0.05$) effects on alkaline phosphatase (ALP) enzyme among all tested groups, however control group showed significantly high concentration value, this in agreed with Asimi and Sahu., (2016), whom indicated that the concentrations of ALT, AST, and ALP enzymes in liver and muscle were decreased in groups fed 0.5 and 1.0% clove extract compared with that of the control group.

Results obtained recorded no significant ($P\geq0.05$) differences for calcium level in blood serum between treated groups compared with control group which had the lowest level. For phosphorus there were significant ($P\leq0.05$) differences among all treated groups, control group had the highest level.

There were no significant differences in commercial cuts percentages, their separable tissue, also chemical composition of meat (Table 33) was not affected significantly by dietary treatments, which was confirmed by the subjective meat quality values. These results were in harmony with findings of Mukhtar (2011); Amal *et al.*, (2013); Daffallaa and Mukhtar, (2016), but in contrast with the report of Alcicek *et al*., (2004), who observed improvement in percentage by the dietary essential oil.

Result of economical evaluation (Table 36), showed that group of chicks fed on 200 mg/kg mixed oils recorded (1,222) as the highest profitability ratio compared with the control group. So addition of dietary mixed essential oils improved the performance of broiler chicks and resulted in economic benefits. Our finding in the same line with Issam, (2018), who found the economic benefits when used dietary essential oil.

5. 4 Experiment (4):

Response of broiler chicks fed on graded levels of Eucalyptus, Clove and Basil mixed essential oils (ECB MEO):

Results of feeding broiler chicks on graded levels of Eucalyptus, Clove and Basil mixed essential oils were found that no significant ($P \geq 0.05$) effects in body weight, feed intake, body weight gain and feed conversion ratio among all tested groups (Table 37), and these results in contrast with several studies which reported beneficial effects of many combinations of essential oils (mix, blends or just oils) on weight gain Bento *et al*., (2013); Péron *et al*., (2009); Yang *et al*., (2009), and agreed with others reported no effect (Vlaicu *et al*., 2017). These differences have been attributed to the type of essential oils used and inclusion level Cross *et al*., (2007), also they were disagreed with saying of Amerah *et al*., (2011), who stated that some essential oils supplementation significantly improved weight gain. On the contrary, several studies have reported that medicinal plants in diets enhance the growth performance in birds. In the same way, Salami *et al*., (2015), reported that use of medicinal herbs in the diets of broiler improved the feed conversion ratio values at the end of the trial. Buchanan *et al*., (2008) stated that broiler chickens fed diets having plant extract blends had minimum feed conversion ratio and had increased weight gain. In general, the improvement found on growth performance was not linearly related to the inclusion levels of mixed essential oils, and the greater performance values were obtained at 200 and 400 mg/kg of mixed essential oils inclusion.

Also results declared no significant effects on the broiler dressing and giblets (liver, heart and gizzard) (Table 38), non-carcass components, commercial cuts and their meat values which were confirmed with the panel taste, all being at moderate values (Table 39, 40, 41, 42).

Glucose results (Table 44), had no significant effects between control group and group supplemented with 200 mg/kg mixed oils compared with groups supplemented with 400 mg/kg and 600 mg/kg mixed oils which had the highest level. Tri-glyceride result showed no significant effects between groups supplemented with 200 mg/kg and 400 mg/kg mixed oils compared with group supplemented with 600 mg/kg mixed oils which had the highest level and control group. Protein result also registered no significant effects between groups supplemented with 200 mg/kg and 600 mg/kg mixed oils compared with group supplemented with 400 mg/kg mixed oils and control group which had the highest level .Albumin result registered no significant effects among control group and groups supplemented with 200 mg/kg and 600 mg/kg mixed oils compared with group supplemented with 400 mg/kg mixed oils which had the lowest level, but cholesterol result revealed significant effects among all tested groups, however control group had the lowest level compared with other supplemented groups and these in contrast with the finding of Fathi *et al*., (2020); Mercati *et al*., (2020) and Placha *et al.*, (2019), whom reported that the addition of mixed essential oils further showed improvements in averaged daily gain, growth performances, carcass quality and reduced cholesterol level in broilers, and the same results obtained for HDL and LDL levels, and for urea control group had the highest level compared with other groups supplemented with mixed essential oils. These findings were in agreement with Mahmoud *et al.,* (2011), study in which the best results in kidney functions, it has been found these treatments decreased the mean values of urea nitrogen due to improvement in the glomerular function of kidney and maintained positive nitrogen balance., for uric acid there were significant effects among all tested groups, group supplemented with 200 mg/kg mixed oils had the highest level compared with group supplemented with 400 mg/kg and group supplemented with 600 mg/kg mixed oils, whereas control group had the lowest level. Creatinine results revealed no significant effects among all groups supplemented with mixed oils compared with control group.

On the other hands no significant effects on aspartate amino transfers (AST) enzyme (Table 45) among groups supplemented with 200 mg/kg, 600 mg/kg and control group, compared with group supplemented with 400 mg/kg mixed oils which showed significantly low concentration value. But there were significant ($P \leq 0.05$) effects on alkaline phosphatase (ALP) enzyme among all tested groups, however control group showed significantly high concentration value, our findings were in contrast with Mustafa., (2016), who showed no significant differences in AST and ALP enzyme concentrations when broiler chickens were fed mixed essential oils (clove, anise, and caraway) at a rate of 200, 400, and 600 mg/kg feed.

Result of calcium level in blood serum revealed no significant effects between control group and group supplemented with 200 mg/kg mixed oils and control group, compared with other supplemented groups, but for phosphorous there were significant effects among all tested groups that control group had the highest whereas group supplemented with 400 mg/kg mixed oils had the lowest level. There was no significant effect in commercial cuts percentages, their separable tissue, also chemical composition of meat was not affected significantly by dietary treatments, which was confirmed by the subjective meat quality values. The result in line with (Mukhtar, 2011; Amal *et al*., 2013).

Result of economical evaluation (Table 46), showed that group of broilers fed on 200 mg/kg mixed oils recorded (1,202) as the highest profitability ratio compared with the control group. So the addition of dietary mixed essential oils improved the performance of broiler chicks and resulted in economic benefits. This result in the same line with Issam, (2018), who found the economic benefits when used dietary essential oil.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6. 1 Conclusion:

From the results obtained from present studies we can concluded that supplementation of graded levels of different combinations of mixed essential oils (Eucalyptus essential oil, Clove essential oil, Basil essential oil) in broiler diets can be suitable as a natural growth promoter without any adverse effects in the performance.

The supplementation of mixed essential oils in graded levels to broiler diets had no significant effects on the performance, however Inclusion of (200 mg/kg) mixed essential oils to diet recorded the highest body weight, body weight gain, feed intake and best feed conversion ratio compared with control group and other combinations.

Inclusion of mixed essential oils in graded levels to broiler diets had no significant effects on carcass and carcass yield, subjective and objective of meat quality, the same results for cholesterol, total protein and uric acid concentration and had decreased effect for urea, but had increased effect in the levels of calcium, while no significant effects in P ratio. AST and ALP enzymes activities.

Good to mention that, adding of mixed essential oils to broiler diets in graded levels had economic benefits.

6. 2 Recommendations:

6. 2. 1 Practical Consequence:

1. According to the results of our experiments, the essential oils extracted from Eucalyptus leaves, Clove fruits and Basil plants and their combination in (1: 1: 1) could be suitable as a natural growth promoter.

2. According to the finding of our experiments, it may be useful to re-considered more combinations to gain more beneficial effects on the performance of the broilers and the other parameters.

6. 2. 2 Recommendations for future researches:

1. More others combinations of another kinds of essential oils are needed to get more complete evaluations for the effects of mixed essential oils in diets on the performance of broilers.

2. Study the effects of the mixed essential oils in the immunity of the layer.

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Appendixes

Appendix (1)

Weekly maximum and minimum experimental temperature by (Cº) in duration from 19 January to 23 February 2019:

Weeks	Minimum	Maximum
1	24	34
2	22	30
3	20	26
4	18	22
5	16	18
Average temperature	20	26

Appendix (2)

Card used for judgment of subjective meat quality adjectives Sensory Evaluation. Evaluate these sample for tenderness, flavor, color and juiciness. For each sample, use the appropriate scale to show your attitude by checking at the point that best describes your feeling about the sample. Any question, please ask. Thank you for participation.

Tenderness	Flavor	Color	Juiciness
8-Extremely tender	8-Extremely intense	8-Extremely desirable	8-Extremely juicy
7-Very tender	7-Very intense	7-Very desirable	7-Very juicy
6-Moderately	6-Moderately intense	6-Moderately	6-Moderately juicy
5-Slightly tender	5-Slightly intense	5-Slightly desirable	5-Slightly juicy
4-Slightly tough	4-Slightly bland	4-Slightly undesirable	4-Slightly dry
3-Moderately tough	3-Moderately bland	3-Moderately undesirable	3-Moderately dry
2-Very tough	2-Very bland	2-Very undesirable	2-Very dry
1-Extremely tough	1-Extremely bland	1-Extremely undesirable	1-Extremely dry

Serial	Sample code	Tenderness	Flavor	Color	Juiciness	Comments
A	1					
B	2					
C	3					
D	4					
E	5					

Appendix (3)

Chemical analysis of Eucalyptus essential oil for chemical composition and actives component.

No	RT(min)	Name	Area Sum%
1	4.77	a-Pinene	1.87
2	486	3-Carene	1.36
3	4.94	a-Phellandrene	0.64
4	5.37	Pseudolimonen	0.53
5	5.81	Cis-2-Menthenol	6.4
6	6.59	Y-Terpinene	1.43
7	6.83	a-Terpinolen	1.53
8	7.06	Isocarveol	0.78
9	7.10	Carveol	0.5
10	7.17	Trans-2-Menthenol	1.56
11	7.35	L-trans-Pinocarveol	1.86
12	7.49	Pinocarvone	0.54
13	7.79	4-Carvomenthenol	4.82
14	8.14	a-Terpinol	0.46
15	8.33	Trans-Pinocarvyl acetate	1.15
16	8.78	D-(+)-Camphor	21.94
17	8.91	Phellandral	0.84
18	9.16	Carvacrol	1.94
19	9.49	Y-Elemene	0.47
20	9.68	Trans-Isoeugenol	0.57
21	9.87	Cubebol	0.74
22	10.00	ß-Elemene	1.78
23	10.20	Aromandendrene	0.58
24	10.29	Caryophyllene	1.01

25	10.50	Gurjunene	4.9
26	10.64	Cis-Lanceol	4.88
27	10.75	(+)-Valencene	1.42
28	10.88	ß-Eudesmene	1.38
29	10.97	a-Guaiene	1.94
30	11.03	a-Copaen-11-o1	0.53
31	11.08	Cis- ß-Copaene	0.58
32	11.12	Nootkatene	1.42
33	11.40	Elemol	6.03
34	11.51	Viridiflorol	0.49
35	11.68	Cedrenol	5.1
36	11.92	Caryophyllene oxide	0.53
37	12.02	a-Eudesmol	1.63
38	12.09	Y-Eudesmol	1.06
39	12.14	a-Cadinol	0.69
40	12.27	ß-Eudesmol	4.4
41	12.38	ß-Costol	0.49
42	12.90	7-Isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-one	4.65
43	13.04	Cembrene	0.57
44	14.54	a-Irone	0.98
45	15.55	Corymbolone	0.52
46	18.88	Phytol	0.46

*source :(Regional Center for Food and Feed, Ministry of Agriculture, Egypt).

Appendix (4)

Chemical analysis of Basil essential oil for chemical composition and actives component.

No	RT(min)	Name	Area sum%
1	4.76	Pseudolimonen	0.6
2	5.28	ß-Pinene	2.17
3	5.47	ß-Myrcene	0.87
4	6.09	Eucalyptol	8.4
5	6.23	3-Carene	1.7
6	6.66	ß-Camphor	0.67
7	7.32	Linalool	15.66
8	7.61	(+)-2-Bornanone	0.66
9	7.97	Chavicol methyl ether	2.47
10	8.25	Anethole	4.53
11	8.55	D-Carvone	0.31
12	8.38	Acorenone B	0.8
13	8.99	Bornyl acetate	0.6
14	9.56	Y- Elemene	0.93
15	10.09	Eugenol methyl ether	21.49
16	10.44	Methylisoeugenol	11.96
17	10.64	Aromandendrene	2.94
18	10.73	Caryophyllene	0.27
19	10.81	a-Himachalene	0.56
20	10.86	Y-Muurolene	0.3
21	11.01	Alloaromadendrene	1.45
22	11.14	a-Guaiene	3.98
23	11.27	Isoledene	2.25
24	11.39	a-Cadinene	0.25
25	11.48	Elemol	0.52

26	11.54	Caryophyllene oxide	0.41
27	11.74	Cedrenol	0.37
28	11.78	Lanceol, cis	0.38
29	11.87	d-Viridiflorol	0.46
30	12.01	Cubebol	1.16
31	12.07	a-Cadinol	0.27
32	12.26	Y-Gurjunene	2.91
33	12.32	ß-Eudesmol	0.83
34	12.42	Isoaromadendrene epoxide	0.48
35	12.49	Humulenol-II	0.52
36	13.62	Phytol	0.32
37	12.89	2(IH)Naphthalenone,3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)	0.43
38	14.82	m-Camphorene	0.35
39	14.97	ß Carotene	1.3
40	15.06	Gardenin	0.55
41	15.73	Quercetin 3,5,7,3',4'-pentamethyl ether	1.13
42	17.66	Dehydrodieugenol	1.00
43	18.66	Orientin	0.8

*source :(Regional Center for Food and Feed, Ministry of Agriculture, Egypt).

Appendix (5)

Chemical analysis of Clove essential oil for chemical composition and actives component.

No	RT(min)	Name	Area Sum%
1	4.80	a-Pinene	0.48
2	5.61	a-Terpinolen	0.59
3	5.91	Eucalyptol	0.87
4	6.78	(+)-cis-Verbenol, acetate	0.64
5	7.23	Linalool	1.08
6	8.41	Methyl salicylate	0.85
7	8.56	3-Carvomenthenone	0.9
8	9.12	Carvenone	1.42
9	9.33	Chavicol	3.24
10	9.60	Isoeugenol	6.9
11	10.40	Eugenol	46.54
12	10.80	a-Humulene	1.67
13	11.10	Aceteugenol	0.79
14	11.56	Trans-Isoeugenol	11.77
15	11.66	ß-Eudesmol	1.54
16	11.93	Caryophylladienol II	1.27
17	12.09	Viridiflorol	1.74
18	12.25	Uncineol	1.01
19	12.30	Cedrelanol	0.81
20	12.42	a-Eudesmol	2.01
21	12.55	2',3',4'Trimethoxyacetophenone	2.02
22	13.62	Epi-Y-Eudesmol	1.26
23	14.63	Methyleugenol	0.52
24	15.56	Schaftoside	0.50
25	16.65	Phytol	0.53

26	17.54	2',3'-Dimethoxyflavanone	0.48
27	17.68	Kaempferol 3,7, 4'-trimethyl ether	2.59
28	17.84	3'-Benzyloxy-5,6,7,4'-tetramethoxyflavone	1.66
29	18.53	Dimethyl-3,7-di(4-methoxyphenyl)-2,8-dioxo-2H,8H-benzo(1,2-b:5,4-b')dipyran	0.57
30	18.67	Isoorientin	0.95
31	19.54	3,7,8,2'-Tetramethoxy flavone	0.71
32	19.78	2',3',7-Trimethoxy flavone	0.59
33	20.10	Bergenin	0.83
34	20.39	6,7,8-Trimethoxycoumarin	0.66

*source :(Regional Center for Food and Feed, Ministry of Agriculture, Egypt).