World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

RESPONSE OF BROILER CHICKS TO GRADED LEVELS OF THREE MIXED ESSENTIAL OILS

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Article Received on 16/03/2022 **Article Revised on 05/04/2022** **Article Accepted on 26/04/2022**

ABSTRACT

This experiment was conducted to evaluate the response of broiler chicks to diets supplemented with graded levels of three mixed essential oils (MEO) in a combination of them, extracted from eucalyptus leaves, clove fruits and basil plants (Eucalyptus Essential Oil, Clove Essential Oil and Basil Essential Oil) (1: 1: 1) as natural feed additives. A total of 96 one day old un-sexed (Cobb) broiler chicks were subjected to 35 days experimental period. Chicks randomly divided into four groups of 24 chicks per each with three replicates for each group. Four experimental diets were formulated (A, B, C and D) as follows, diet A as control, diet B, C and D was control diet supplemented with mixed essential oils (MEO) at 200, 400 and 600 mg/kg respectively. The experiment parameters covered carcass and non- carcass values, serum constituents, enzyme activities and economical appraisal. The results indicated that there were no significant differences among all tested groups in internal organs, commercial cuts, their separable tissues and the subjective and objective of meat quality parameters. The results of serum metabolites showed that inclusion of (MEO) at graded levels significantly increased the glucose level, whereas protein level less than control, also for cholesterol. Urea level more high in control and uric acid less than in inclusion of (MEO),also the results of enzymes activities declared that inclusion of (MEO) at graded levels significantly reduced the levels of AST and ALP enzymes in the blood, the result of theeconomical evaluation of (MEO) in broiler diets showed economicefficiency. That200mg/kg (MEO) recorded (1.202)the highest profitability ratio than all other groups.

KEYWORDS: Eucalyptusessential oil, serum metabolite and AST, ALP.

INTRODUCTION

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 pathogenicmicro 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 essential oils have a great potential. The essential oils are generally considered natural, and free from residues when compared with antibiotics (Gong *et al*., 2014).

Essential oils are complex mixtures of volatile compounds produced by living organisms and isolated by physical means only(pressing and distillation) from a whole plant or plant part ofknown taxonomic origin (Franz and Novak., 2009). The term"essential oils" emerged because "oils" were wishfully believed tobe "essential" to life, and have a long history of being used by humanfor cosmetic and medicinal purposes. Essential oils, are usually utilized in animal feeding and are considered growth and immune enhancers due to their anti-oxidant, anti-microbial and digestion properties (Abdulkarimi *et al*., 2011; Assiri *etal*., 2016), their stimulating on animal performance (Ciftci*et al*., 2005), digestive enzymes (Lee *et al*., 2002; Jamroz and Kamel., 2002) and improve utilization of digestive products through enhanced liver function (Hernandez *et al*., 2004), as well as inhibition of odors and ammonia control (Varel., 2002). The use of essential oils is gaining much attention in modern livestock and poultry production systems because of having multi-dimensional benefits with the ultimate objective of maintaining overall health (Idris *et al*., 2017; Abbas *et al*., 2018; Khater *et al*., 2018; Ahmad *et al*., 2019; Fayaz *et al*., 2019).

Several studies have reported beneficial effects of many combinations of essential oils (mix, blends or just oils) on weight gain (Bento *et al*., 2013), while 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).

Eucalyptus is a medicinal plant that belongs to Myrtaceae family, originated in Australia but found worldwide, especially in tropical and sub-tropicalregions (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). 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).

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. 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 insmaller 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.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 andanti-oxidant effects (Najafi and Torki., 2010). 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).

Basil(Ocimum basilicum L.) is an annual plant of the Lamiaceae family, growing wild in sub-tropical and tropical areas of America, Africa, Asia, and in some southern regions inEurope (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). 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).

MATERIALS AND METHODS

This experiment was conducted at Poultry Production Farm, College of Agricultural Studies, Sudan University of Science and Technology during the period from 19 January to 23 February 2019, in winter season which ambient temperature average 20-26 Cº.

A total of 96 one day old un-sexed broiler chicks (Cobb strain) were purchased from commercial company in Khartoum. The chicks were adapted to the premises for one week and feed 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 New-

castle disease by Avinew–France respectively. Combinations of AD3pantominovite–pantex Holand and B.V. 5525 ZGDuize lHoland. As a soluble multi-vitamins was provided three days before and after vaccinationprograms in order to guard stress.

The experimental house in which the chicks were kept was semi closed. 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 sawdust (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.

For essential oil, Eucalyptus, Clove and Basil were submitted to hydro distribution using n-hexane as a collecting solvent. The solvent was removed under vacuum and the quantities of theessential oils were determined by gas chromatography, at Industrial Research Center, Khartoum North. And they were mixed at (1:1:1) ratio to be used as a natural growthpromoter. Four experimental diets were formulated to meet the requirements of broilers chicks according toNutritional Research Council (NRC 1994). 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 and basil mixed essential oils as growth promoter, at levels of 200, 400, 600 mg/kg feed respectively. Experimental diets were fed for five weeks.

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 rate recorded daily.

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, kidney, gizzard, abdominal fat and intestine) and then were separated weighed individually and were expressedas apercentage of live weight. The hot carcass were weighed to calculate the dressing percentage. And 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 aspercentage of their cuts. The meat was frozen and stored for meat analysis.

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 approximately80 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.

Completely randomized design used in this experiment. Data collected were supplied to analysis of variance (ANOVA) means were further separated by Dun cans multiple range test(1955), the level of significant difference set up at ($p<0.05$).

RESULTS

The specific chemical constituents of oils determined by testifying oil distasted from eucalypts leaves showed nine main chemical compounds: D-(+)-Camphor, Cis-2-Menthenol, Elemol, Cedrenol, Gurjunene, Cis-Lanceol, 4-Carvomenthenol, 7-Isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-one, ß-Eudesmol.

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

For body weight there were no significant ($P\geq0.05$) differences observed between all tested groups, however essential mixed oils at all levels improved body weight, body weight gain and feed conversion ratio, however group of chicks fed on 600 mg/kg mixed oils showed the best values for the above parameters compared to control group.

The results of adding graded levels of Eucalyptus and Clove and Basil mixed essential oils on non-carcass were showed in table (2). There were no significant ($P\geq0.05$) differences observed between all tested groups for non-carcass.

Table 1: Effect of adding graded levels of Eucalyptus and Clove and Basil mixed essential oils on performance of broiler chicks.

Treatments	BW	FI	BWG	FCR
Control	1942.0	3428.7	1757.0	1.950
200 mg/kg	1979.7	3437.7	1794.7	1.913
400 mg/kg	2074.7	3399.0	1889.7	1.813
600 mg/kg	2045.7	3342.3	1860.7	1.810
SE±	137.88	143.87	137.88	0.135

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 FCR; Feed conversion ratio BWG; Body weight gain(g), FI; Feed intake (g) BW; Body weight (g)

Table 2: Effect of adding graded levels of Eucalyptus and Clove and Basil mixed essential oils on Non-carcass.

Treatments	Lung %	Kidney %	Abdominal fat%	Intestine weight%	Intestine length%	Back %	Wing %
Control	0.727	0.373	1.067	3.800	178.33	19.693	10.467
200 mg/kg	0.473	0.427	0.757	3.500	184.67	20.533	10.200
400 mg/kg	0.583	0.463	1.187	3.470	183.00	17.733	10.533
600 mg/kg	0.643	0.477	0.957	3.730	172.33	20.300	10.400
SE±	0.116	0.058	0.155	0.296	7.394	0.916	0.559

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

The results of feeding graded levels of Eucalyptus and Clove and Basil mixed essential oils on commercial cuts were showed in table (3). No significant (P≥0.05) differences among all tested groups for commercial cuts. Also no significant (P≥0.05) differences among all tested groups for meat values.

Table 3: Effect of adding graded levels of Eucalyptus and 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

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

Chicks fed on 600 mg/kg mixed oils showed significantly highest (P≥0.05) glucose concentration value compared with other tested groups (table4), at the same time this group recorded significantly the highest values for tri-glyceride concentration compared with the other tested groups.Chicks fed on 400 mg/kg mixed oils showed the lowest albumin concentration compared with other tested groups, at the same time this group recorded significantly the lowest cholesterol HDL values. Chicks fed on 200 mg/kg mixed oils showed significantly highest (P≥0.05) cholesterol concentration value compared with other tested groups, at the same time this group recorded significantly the highest values for cholesterol HDL and cholesterol LDL concentration compared with the other tested groups.Chicks fed on control diet showed the highest urea concentration compared with the other tested groups, whereas the same group showed the lowest uric acid and creatinine concentration compared with the other tested groups.

Chicks fed on 400 mg/kg mixed oils showed significantly lowest (P≥0.05) AST enzyme concentration in the blood compared with other tested groups, at the same time this group recorded significantly the lowest ratios for Ca^{++} andPO4 concentration compared with the other tested groups. Chicks fed on 200 mg/kg mixed oils showed significantly lowest (P≥0.05) ALP enzyme concentration in the blood compared with other tested groups, whereas control group recorded the highest one.

Profitability ratio (1.202) of group of chicks fed on 200 mg/kg mixed oils was the highest of the tested groups

Table 4: Effect of adding graded levels of Eucalyptus and 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)	Urea (mg/dl)	Uric acid (mg/dl)	Creatine (mg/dl)	AST (iu/l)	ALP (iu/l)	Ca (mg/dl)	Po (mg/dl)
Control	220.50^{c}	43.500^{b}	3.950^{a}	2.050^{a}	124.50^{d}	7.100^{a}	3.467^{d}	2.110^{c}	28.950^{a}	247.50^{a}	8.150^{a}	8.750^{a}
200 mg/kg	223.50^{c}	39.500^{c}	2.550^{c}	1.900^{ab}	143.50^{a}	4.500^{b}	6.667^{a}	2.440^{a}	29.550^{a}	165.25^{d}	8.150^{a}	7.150^{b}
400 mg/kg	229.00^{b}	40.500^{c}	2.950^{b}	1.600^{c}	130.00^{c}	4.000^{bc}	5.400^{b}	2.300^{b}	25.950^{b}	183.00^{c}	5.900^{c}	5.650^{d}
600 mg/kg	233.00^{a}	50.500^{a}	2.700^{c}	1.800^{b}	141.00^{b}	3.500^{c}	4.600^{c}	2.420^{ab}	29.450^{a}	197.10^{b}	7.400^{b}	5.950^{c}
SE±	1.118	0.408	0.054	0.061	0.646	0.292	0.133	0.043	0.394	0.928	0.087	0.091

Values are mean± SDMeans value(s) bearing no different superscript(s) in a column are not significantly different (P≥0.05) according to DMRTSE ± Standard Error

DISCUSSION

Chemical analyses of eucalyptus leaves essential oil reported that,D-(+)-Camphor, Cis-2-Menthenol, Elemol, Cedrenol, Gurjunene, Cis-Lanceol, 4-Carvomenthenol, 7-Isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-one, ß-Eudesmolwere the main compounds. These results were in contrast with the finding of (Bugarin *et al*., 2014), who said1,8 Cineole was confirmed as the main component of eucalyptusessential oil.However, (Khaled *et al*., 2015) identified monoterpene hydrocarbons as the major constituents. These variations in the main compounds might be due to the differentiation and the age of the plants, and the chemical composition of essential oils depends on species, topographical location, harvesting stage, parts of the plant, and extraction methods (Puvača *et al*., 2019).

Results obtained declared no significant effects on the commercial cuts and their meat yield also had no effect on the internal organs (liver, gizzard and heart).

Results declared that supplementation of broiler chicks with graded levels of mixed essential oils significantly decreased the concentration of urea, total protein, and AST and ALP levels, whereas glucose, cholesterol, cholesterol LDL, uric acid and creatinine significantly lower in control group, whilegroup fed on diet supplemented with 400 mg/kg mixed essential oils recorded significantly the lowest values of albumin, cholesterol HDL, Caand PO4.

It should be mentioned that all the results above within normal range, so addition of mixed essential oils in graded levels to dietary of broiler ration, not only can act as growth promoters, but also can substitute for the others one had side effects, without any adverse.

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