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EFFECT OF MIXTURE OF TWO HERBAL ESSENTIAL OILS ON PERFORMANCE, CARCASS TRAITS AND BLOOD SERUM CONSTITUENTS OF BROILER CHICKS

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ABSTRACT

The experiment was conducted to evaluate the response of broiler chicks to diets supplemented with graded levels of mixed essential oils (Clove, Basil) (1:1) on the performance, carcass traits and blood serum constituents of broiler chicks. 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, diets B, C and D were control diet supplemented with mixed essential oils (MEO)at 200, 400 and 600 mg/kg respectively. Result obtained declared that no significant difference in the performance parameters (body weight, feed intake, body weight gain and feed conversion ratio). However group of chicks fed on 400 mg/kg mixed essential oils recorded the highest values. Result also declared no significant difference in dressing percentage, giblets, commercial cuts, subjective and objective of meat quality parameters. Also results for serum constituents declared that, addition of mixed essential oilsreduced cholesterol, cholesterol HDL, cholesterol LDL, total protein and glucose level in the blood compared with control group, whereas urea higher in control group but uric acid lower. the addition of 200 mg/kg mixed essential oils recorded economic benefits. So the study declared that the addition of mixed essential oils as growth promoter in broiler diets have no adverse effects.

KEYWORDS: Clove, Basil, body weight, feed intake, body weight gain and feed conversion ratio.

INTRODUCTION

The poultry industry is very important economically in many countries such as our country Sudan. Most 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 banned using of antibiotics because of increased concern about antibiotic-resistant bacteria and antibiotic residue 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; 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; Jalal *et al*., 2019).

Clove extract and exhibits wide range of anti-microbial activity in vitro (Ehrich *etal*., 1995) in addition, anti-septic, appetite and digestion stimulant (Kamel *et al*., 2001) strong anti-microbial and anti-fungal (Ehrich *etal*., 1995), anti-parasitic (Kim *etal*., 2004) and anti -oxidant (Dargland *etal.,* 2003), activities of clove and its ingredients have been reported. many of the studies have reported that clove was rich in trace mineral which essential for protein and carbohydrate metabolism. Reduced the synthesis of fatty acid and cholesterol that could be improved broiler performance (Hernaddez *et al*., 2004).

Activities of basil essential oils have been established as anti-oxidant, anti-microbial, anti-inflammatory, anti-bacterial, anti-fungal activities as well as repellent,

insecticidal, larvicidal and nematicidal (Avetisyan *et al*., 2017; Kavoosi *et al*., 2017; Saggiorato *et al*., 2012).

The objective of our present study was to examine the effect of dietary supplementation of an herbal mixed essential oil (MEO) on growth performance, carcass traits and blood serum constituents of broiler chicks.

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 temperatureaverage 20-26 C°.

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 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 Gumbo best 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 AD3 pantominovite – pantex Holand and B.V. 5525 ZG DuizelHoland. As a soluble multi- vitamins was provided three days before and after vaccinationprograms in order to guard stress.

Chicks were kept was semi closed house, 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 enablesthe 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 shaves (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 Clove and Basilwere 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) ratio to be used as a natural growth promoter. 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 clove and basil mixed essential oils as growth promoter, at levels of 200, 400, 600 mg/kg feed respectively.The ingredients percent composition were calculated and the chemical composition of the experimental control diet were presented in table (1). Experimental diets were fed for five weeks.

Table 1: Ingredients, Calculated and Chemical Composition of control diet.

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

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

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

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 expressed as a percentage 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 as percentage of their cuts. The meat was frozen and stored for meat analysis.

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: Aspartate Amino Transferase (AST), Alkaline Phosphatase (ALP) and minerals (Ca, P).

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.

Data of performance, carcass traits, 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 ($P \leq 0.05$).

RESULTS

The performance results of broiler chicks fed on diets containing graded levels of Clove and Basil (CB) mixed essential oils were illustrated in **table** (2). Result obtained showed no significant differences ($P \geq 0.05$) in the performance parameters: (Body weight, feed intake, body weight gain and feed conversion ratio).There were no significant ($P \geq 0.05$) differences observed among all tested groups for body weight and body weight gain, however groups fed on treated dietary recorded heavy weight compared to control group, while chicks fed on 400 mg/kg mixed oils had the heaviest body weight compared with other tested groups. For feed intake no significant ($P \geq 0.05$) differences were noticed, but group of chicks fed on 200 mg/kg mixed oils consumed more compared with dietary groups, for feed conversion ratio, observed significant ($P \geq$ **0**.05) difference between tested groups of chicks, however, group of chicks fed on 400 mg/kg mixed oils had the best feed conversion ratio. Result declared no significant difference ($P > 0.05$) among all treated groups in the percentages of carcass dressing and giblets, commercial cuts and their separable tissue,

meat chemical composition and subjective meat quality parameters(table3).

Table 2: Effect of adding graded levels of Clove and Basil mixed essential oils on the performance of broiler chicks.

Treatments	Body weight (g)	Feed intake (g)	Body weight gain (g)	Feed conversion ratio
Control	1942.0[a]	3428.7a	1757.0a	1.950[a]
200 mg/kg	2129.3a	3444.0a	1944.3a	1.770[ab]
400 mg/kg	2145.0a	3319.0a	1960.0a	1.700[b]
600 mg/kg	2068.3a	3301.0a	1883.3a	1.760[ab]
SE±	123.97	116.26	123.97	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 Duncan's Multiple Range Test (DMRT). SE ± Standard Error

Table 3: Effect of adding graded levels of Clove and Basil mixed essential oils on dressing And giblets (liver, heart and gizzard) commercial cuts and.

Treatments	Dressing%	Breast%	Drumstick%	Thigh%	Liver%	Heart%	Gizzard%
Control	70.310	39.460	11.730	15.067	2.047	0.510	1.547
200 mg/kg	70.680	37.613	11.717	13.487	2.230	0.603	1.437
400 mg/kg	68.320	37.633	12.973	14.493	2.037	0.537	1.390
600 mg/kg	70.457	36.853	12.157	13.897	1.803	0.503	1.340
SE±	1.014	1.427	1.254	1.207	0.179	0.090	0.210

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

The results of adding graded levels of Clove and Basil mixed essential oils on meat chemical composition were showed in table (4). Moisture, ether extract and crude protein contents decreased with the increase of level of the mixed essential oil in the diet. Group of chicks fed on 600 mg/kg mixed oils had the lowest values for dry matter, ash and ether extract.

And for serum metabolites, **table** (5) declared that, for glucose level there were significant (**P≥0.05**) difference between tested groups, however, chicks fed on control diet and 200 mg/kg mixed oils recorded the highest level, while those fed on 400 mg/kg mixed oils had the lowest level, and for triglyceride there was significant (**P≥0.05**) difference between tested groups, however results showed that inclusion of mixed essential oil decreased significantly (P≥0.05) the level of triglyceride.

Also for total protein, albumin and urea result obtained showed significant (P≥0.05) decreased for their levels between treated group of chicks compared to control group.

Also for cholesterol no significant (P≥0.05) differences were observed between group of chicks fed on control diet, 400 mg/kg mixed oils and those fed on 600 mg/kg mixed oils compared with group of chicks fed on 200 mg/kg mixed oils which had the lowest level, the level of serum urea showed significant (P≥0.05) difference between group of chicks, to broiler diet significant (P≥0.05) decreased the level of urea while the opposite result recorded for uric acid. finally for creatinine result showed significant (P≥0.05) difference between groups of chicks, however, control group revealed the lowest value while group of chicks fed on 600 mg/kg mixed oils had the highest level.

On the other hands, economically the addition of mixed essential oils as a natural growth promoter at graded levels resulted in economic benefits; obviously the addition of mixed essential oils at 200 mg/kg as growth promoter in broiler diet recorded the highest profitability ratio compared with the control and even all others supplemented groups.

Table 4: 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 Duncan's Multiple Range Test (DMRT). SE ± Standard Error

Table 5: 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
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

DISCUSSION

Result showed no effect on the mortality rate of experimental chicks due to treatments, the rate of mortality was lower than commercial situation, this might be due to the good hygiene conditions, and it could be suggested that the actives substances contained in the mixed essential oils productmight have had a positive effect in gut micro flora which reduce the amount of pathogenic bacteria. This result was in line with the finding of (Mukhtar. 2011, Mukhtar *et al.,* 2013). Alsoresults of feeding broiler chicks on graded levels of Clove and Basil mixed essential oils were found that no significant (P≥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 said no significant differences in growth performance (BWG and FCR) were observed when broiler chickens were fed clove bud supplements at a rate of 0.5 and 1.0 g/kg diet.Result observed no significant effects on the broiler dressing and giblets, commercial cuts and their separable tissue percentages, meat chemical composition which was confirmed with the panel taste values. The results were in line with founding's of (Mukhtar, 2011), but not agreed with the report of (Alcicek*et al*., 2004) who observed improvement in dressing percentage by the dietary essential oil. Glucose results for chicks supplemented with 400 and 600 mg/kg mixed oils registered significant low concentration level, and this was agreed with the finding of (Baker *et al*.,2008), who reported that use of medicinal plants by stimulating insulin secretion and prevent cellular resistance to insulin, reduced blood glucose levels, andfor 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 and control group which had the lowest, this variation might be due to the concentration of the essential oils.

From the result obtained it can be concluded that mixed essential oils (Clove, Basil) can be used as an alternative natural growth promoter substance for broiler dietary without any adverse effect.

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