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Performances of Broiler Chickens Fed on Diet Supplemented with Thyme and Oregano Essential Oils Stabilized in a Plant Charcoal Matrix

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ABSTRACT

This study was designed to mitigate the volatile and oxidative ability of essential oils (EOs) in poultry feed using natural plant charcoal. The dietary treatments consisted of supplementing control diet (R0) with 0.01% of the mixture (1/1) of thyme and oregano EOs (R_{Th+or}), 0.2% of *Canarium* charcoal without EO (R_{0C}), 0.2% charcoal respectively enriched with 0.01% of thyme EO (R_{0C+Th}), oregano EO (R_{0C+Or}) and the mixture of EOs ($R_{0C+Th+Or}$). Results revealed a non-significant increase in weight gain for about 5 and 6%, respectively with the mixture of the EOs without charcoal and charcoal enriched with the mixture of the EOs compared to the control (R_0). The carcass yield was higher with oregano EO and the mixture of EOs compared to the other treatments. Intestinal density was lower ($P<0.05$) with the mixture of the EOs compared to thyme EO alone and the control ration. Charcoal containing EOs significantly increased ($P<0.05$) total protein in serum content, triglycerides, albumin, globulin and decreased serum content in creatinin, ASAT, ALAT and cholesterol. Hematological parameters were not significantly affected by the treatments. The blend of EOs associated or not to charcoal increased lactic acid bacteria count in both the ileum and the cæcum as compared to *E. coli* and salmonella. It was concluded that *Canarium* charcoal can be used to stabilize EOs in the feed for gut microbiota modulation and better growth performances of broiler chickens.

Key words: Broiler, Essential oil, Hematology, Gut microbiota, Oregano, Plant charcoal, Thyme

INTRODUCTION

Antibiotic Growth Promoters (AGPs) have been banned in several countries of the world because of the resistances developed by bacteria and the presence of the chemical residues in the livestock products which could have harmful consequences for the consumers (Vicente et al., 2007). Many alternatives to the AGPs have been identified and are already sold as food additives for livestock. Among these alternatives we can list the probiotics, prébiotics, organic acids, plants extracts and Essential Oils (EOs).

Essential oils are odorous compounds, generally of complex composition that give plants their color and scent. The studies of Bolukbasi et al. (2006), Cross et al. (2007) and Khattak et al. (2014) revealed that EOs stimulated feed intake, improve weight gain and offer health advantages to poultry. One of the main mechanisms which explain the improvement of poultry performances is the ability of EOs to balance the gut microbiota by inhibiting pathogens bacterial growth due to their antibacterial properties. The antibacterial activity of EOs is due to the presence of several active compounds such as the carvacrol, thymol, eugenol or cinnamaldéhyde (Mathlouthi et al., 2009; Al-Shuwaili et al., 2015; Moukette et al., 2015). These compounds interact with and increase the permeability of the bacteria cell membrane, deteriorate the enzymatic systems and inhibit or destroy the genetic material of the bacteria (Hulin et al., 1998; Krishan and Narang, 2014). Another responsible mechanism could be the stimulation of feed, and the secretion of the digestive enzymes of the host (Bento et al., 2013), and the modulation of the immune system which offer healthy performance benefits to poultry (Brenes and Roura, 2010; Tiihonen et al., 2010; Amerah et al., 2011; Hosseini et al., 2013; Khattak et al., 2014; Karadas et al., 2014). *In vitro* studies have concluded that the combination of individual EOs has a greater antibacterial effect than individual EOs alone, indicating a synergy between essential oils of different composition and origin (Bento et al., 2013).

Despite all the beneficial properties mentioned above, using EOs in animal feed are still very problematic due to their instability. Their active compounds are volatile and large quantities of EOs are easily lost during feed processing and storage. Incorporation in a stable matrix can overcome the technical issues of stability in feed. Moreover, incorporation in a stable matrix can help to avoid degradative reactions and the loss of EOs quality leading to flavor optimization of feed, better handling, increase stability, delay release and then enhance the bioavailability of EOs in the digestive tract. The activated charcoal has the ability to bind a variety of substances. This property can be exploited to stabilize essential oils in animal feed, to facilitate their transport and their release in the target sites along the digestive tract where they can optimize the development of beneficial bacteria to chicken. According to Kana et al. (2011), the inclusion of 0.2% of *Canarium schweinfurthii* seeds charcoal in feed increased the weight of broiler chickens for about 12%. The growth promoting effect of this charcoal can be more significant when associated with essential oils.

The present is proposed to give an overview on the potential of plant charcoal to retain and protect essential oil bioactivity, and to evaluate the benefits of the association charcoal-essential oils towards the support of a positive gut bacteria growth and production performances of broiler chickens.

MATERIALS AND METHODS

Site of Study

The study was conducted at the poultry unit of the Teaching and Research Farm of the University of Dschang, Cameroon. This farm is located at 5°26' North and 10°26' EST and at an altitude of 1420 m above sea level. Annual temperatures vary between 10°C and 25°C. Rainfall ranges from 1500-2000 mm per annum over a 9 months rainy season (March to November).

Ethical approval

This study was carried out in strict accordance with the recommendations of institutional guidelines for the care and use of laboratory animals. Chickens were humanly handled in respect of the ethical standards laid down in 1964 Declaration of Helsinki and its later amendments.

Essential oils and charcoal

Essential oils of thyme and oregano were obtained from Barij Esans Company, Tehran, Iran. Mature black fruit seeds (*Canarium schweinfurthii* Engl.) were collected in the villages around the experimental University farm. These seeds were burnt on a wire netting using firewood to obtain black charcoal, it was then quenched with water and sun-dried.

After drying, the charcoal was grounded and sieved to pass through a 1-mm mesh, and lastly used to bind and stabilize essential oils in the experimental rations as follow: Oregano (10g) and Thyme (10g) EOs or their mixture (5g/5g) was respectively mixed with 10g of tween 20 and introduced in 100ml sterile distilled water under constant stirring. After 10 minutess of stirring, 200mg of charcoal was introduced in this solution (EO + tween 20 + distilled water) and homogenized by hand shaking with the hand for 5 minutes to allow the charcoal to absorb all the solution. This EO loaded-charcoal was dried at 55°C for 48 hours and sealed in a nylon bag to prevent air exchanges between the charcoal and the storage environment. This EO loaded-charcoal was used as feed additive with the final EO concentration of 0.01% (10g of EO/100kg of feed).

Animal

320 days-old Cobb500 broiler chicks were acquired from a local hatchery and divided into 5 experimental groups of 64 chicks each. Each group was subdivided into four replicates of 16 chicks (8 males and 8 females in each replicate). Chicks were litter-brooded to 21 days of age at a density of 20 chicks/m². Vaccination and other routine poultry management practices were maintained. Chicks were weighed at the beginning of the experiment and on a weekly basis thereafter. Feed and water were offered *ad libitum.*

Dietary treatments and experimental design

At both the starter and finisher phases, a control diet (R0) was formulated (Table 1). The dietary treatments consisted of supplementing control diet (R0) with 0.01% of the mixture (1/1) of thyme and oregano EOs (R_{Th+or}), 0.2% of *Canarium charcoal* without EO (R_{0C}), 0.2%

charcoal respectively enriched with 0.01% of thyme EO (R_{0C+Th}), oregano EO (R_{0C+Or}) and the mixture of EOs ($R_{0C+Th+Or}$). Each experimental ration including the control was fed to 16 chicks (8 males and 8 females) replicated 4 times (4 experimental units) chosen at random in a completely randomized design with 5 treatments.

Table 1. Composition of experimental diets

Ingredients (g/kg)	Starter	Finisher
Maize	54	64
Wheat bran	5	1
Soybean meal	22	16
Coton seed meal	5	5
Fish meal	5	5
Borne meal	1	1
Oeister shell	1	1
Palm oil	2	2
Premix 5%*	5	5
Calculated chemical composition		
Metabolizable energy (kcal/kg)	2928.66	3042.76
Crude Protein (g/kg)	23.00	20.40
Lysine (g/kg)	1.43	1.19
Methionine (g/kg)	0.48	0.44
Calcium (g/kg)	1.17	1.35
Phosphorous (g/kg)	0.53	0.56
Crude fibre (g/kg)	5.20	5.14

*Premix 5%: crude proteins 400mg, Lysin 33mg, Methionin 24 mg, Calcium 80 mg, Phosphorous 20.5 mg, metabolizable energy 2078kcal/kg, Vitamins: Retinol 10 000 000 IU, Cholecalciferol 3 000 000 UI, Tocopherol 2500 IU, Phylloquinon 4000 mg, Thiamin 5000 mg, Riboflavin 500 mg, Pyridoxin 2500 mg, Cyanocobalamin 5 mg, Folic acid 10 000 mg and Niacin 2000 mg.

Growth, serum biochemical and hematological parameters

Feed intake, weight gain and feed conversion ration were evaluated on a weekly basis in both starter and finisher phases of the study. At the end of the feeding trial at 49 days of age, 10 birds (5 males and 5 females) from each treatment group were randomly selected, fasted for 24 hours and slaughtered for carcass evaluation as preceded by Kana et al. (2017). From each slaughtered chicken, blood was collected in 02 test tubes, one of which contained an anticoagulant. Blood with anticoagulant was used for the hematological analysis using Genius electronic hematocymeter (Model KT-6180, S/N 701106101557, Hong Kong, China). Hematological parameters included White Blood Cell (WBC), Red Blood Cell (RBC), Haemoglobin (HB), Haematocrit (HCT) and Platelets (PLT). Meanwhile, after centrifugation of blood free from anticoagulant, serum was collected and preserved at -20°C for the evaluation of biochemical parameters (total protein, albumin, globulin, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), total cholesterol, cholesterol HDL and LDL, triglyceride, urea and creatinin) using colorimetric method as prescribed by the commercial kits (Chronolab® kits).

Microbial count

After slaughtering, the ileum and the cæcum from four birds were sampled per treatment and pooled by intestinal segment. The numbers of lactic acid bacteria, *Escherichia coli* and *Salmonella* were counted on appropriate specific culture medium (MRS Agar for lactic acid bacteria, Mac Conkey AGAR for *E. coli* and SS AGAR for *Salmonella* respectively) as proceeded by Pineda et al. (2012).

Statistical analysis

All the data were submitted for analysis of the variance using Statistical Package for Social Science (SPSS 21.0) software. Significant differences between treatment means were indicated using Duncan's multiple range tests at 5 % threshold significance (Vilain, 1999).

RESULTS

Feed Intake (FI), Live Body Weight (LBW), Weight Gain (WG) and gain/feed ratio as affected by thyme and oregano EO and their mixture stabilized in *Canarium* charcoal are summarized in table 2. Feed intake was not markedly affected by the treatments during the starter phase. Supplementing the diet with the mixture of EOs and the mixture of EOs mixed with charcoal resulted in a non significant increase of WG for about 5 and 6%, respectively. The mixture of EOs also induced a significant decrease ($P < 0.05$) in gain/feed ratio compared to the diet supplemented with charcoal without any EOs and charcoal enriched with oregano EO.

The effects of the various treatments on the carcass yield and the relative weight of organs and cut-out are summarized in table 3. Apart for the relative weight of abdominal fat which significantly decreased with charcoal enriched with the EOs or not, treatments failed to induce any marked effect on carcass yield and the relative weight of legs, head, heart and liver.

Table 4 summarized the development of digestive organs of chickens as affected by charcoal enriched with thyme and oregano EOs. Supplementing the diet with charcoal enriched with thyme EO and the mixture of the EOs lead to a significant decrease ($P < 0.05$) in the relative weight of the pancreas. Charcoal enriched with the mixture of the EOs also induced a significant decrease ($P< 0.05$) in intestinal density. Intestine length and density (weight/length) were not markedly affected by charcoal and EOs.

The effect of experimental diets on the microbial load in the ileum and cæcum are summarized in table 5. Irrespective to the bacterial species, bacterial count markedly increased with charcoal without EO and charcoal enriched with the EOs in both the ileum and the cæcum compared to the control diet. Regarding the bacterial species, the feeding of broilers with charcoal enriched with thyme and oregano EOs resulted in a significant increase in the number of lactic acid bacteria colonies as compared to *salmonella* and *E. coli* in the cæcum. In the ileum, the increase in lactic acid bacteria count also reached statistical significance with the mixture of EOs.

Table 6 summarized the effect of enrichment of charcoal with EOs on the serum biochemical parameters. Apart for the urea content which was not markedly affected, experimental diets significantly affected all the studied biochemical parameters. Feeding broilers with charcoal without EO and charcoal enriched with Eos resulted in a marked decrease in ALAT and creatinine. The reverse tendency was recorded in the total cholesterol, triglyceride, total protein, albumin and globulin content which significantly ($P < 0.05$) increased with charcoal alone and charcoal enriched with EOs.

Table 7 indicates the effect of experimental diets on hematological parameters of broiler chickens at 49 days of age. As shown in the mentioned table, the hematological values recorded in the present study indicates no significant ($P > 0.05$) impact of charcoal and EOs on red and white blood cell counts, hemoglobin content and hematocrit percentage.

Table 2. Growth performances of broiler chickens as affected by thyme and oregano essential oils stabilized in *Canarium* seeds' charcoal from one to 49 days old

Study periods (days)	Treatments							
	R_0	R_{Th+Or}	R_{0C}	R_{0C+Th}	R_{0C+Or}	$R_{0C+Th+Or}$	SEM	P
Feed intake (g)								
01 - 21	1416.40^a	1347.83^a	1428.39^a	1379.09^a	1426.80^a	1422.93^a	12.95	0.39
22 - 49	4038.70^b	4460.02^a	4473.86^a	4505.32^a	4592.40^a	4353.52^a	48.02	0.00
01 - 49	5455.10^b	5807.85^a	5873.38^a	5869.34^a	5952.30^a	5711.52^{ab}	118.59	0.01
Live body weight (g)								
01 - 21	751.72^b	808.12^a	781.60^a	785.94^a	796.46^a	795.48^a	6.02	0.10
01 - 49	2657.4^a	2799.27^a	2714.67^a	2741.22^a	2762.97^a	2813.10^a	21.17	0.31
Body weight gain (g)								
01 - 21	709.72^b	766.12^a	739.60^a	743.94^a	754.46^a	753.48^a	6.02	0.10
22 - 49	1905.71^a	1991.14^a	1933.07^a	1955.28^a	1966.51^a	2016.83^a	18.34	0.60
01 - 49	2615.43^a	2757.27^a	2672.67^a	2699.22^a	2720.9^a	2770.31^a	21.17	0.31
Gain/feed ratio								
01 - 21	2.00^a	1.76^b	1.90^{ab}	1.83^b	1.80^b	1.80^b	0.024	0.04
22 - 49	2.12^c	2.24^{ab}	2.32^{ab}	2.31^{ab}	2.34^a	2.16^c	0.025	0.05
01 - 49	2.09^{ab}	2.10^{ab}	2.20^a	2.18^{ab}	2.19^a	2.06^b	0.017	0.08

a.b.c: Means with the same superscript on the same line are not significantly different (P>0.05). P= probability. R_0=control ration without any supplement; R_{Th+Or}=R_0+0.01% mixture of EOs; R_{0C} = R_0 + 0.2% charcoal; R_{0C+Th} = R_0 + 0.2% charcoal + 0.01% Thyme EO; R_{0C+Or}= R_0 + 0.2% + 0.01% Oregano EO; $R_{0C+Th+Or}$= R_0 + 0.2% of charcoal + 0.01% mixture of EOs.

Table 3. Carcasses yield (%) and the relative weight of organs and cuts out (%) of broiler chicken as affected by thyme and oregano essential oils stabilized in *Canarium* seeds' charcoal at 49 days old

Carcass traits (%)	**Treatments**							
	R_0	**R_{Th+Or}**	**R_{0C}**	**R_{0C+Th}**	**R_{0C+Or}**	**$R_{0C+Th+Or}$**	**SEM**	**P**
Carcasse yield (%)	73.09^{b}	74.48^{ab}	73.57^{ab}	73.8^{ab}	74.4^{ab}	74.73^{a}	0.21	0.16
Legs (%BW)	3.45^{a}	3.80^{a}	3.64^{a}	3.56^{a}	3.54^{a}	3.80^{a}	0.06	0.62
Head (%BW)	1.92^{a}	1.84^{a}	1.98^{a}	1.86^{a}	1.95^{a}	1.93^{a}	0.02	0.78
Heart (%BW)	0.51^{a}	0.53^{a}	0.53^{a}	0.47^{ab}	0.39^{b}	0.50^{a}	0.02	0.07
Liver (%BW)	1.67^{a}	1.44^{a}	1.49^{a}	1.55^{a}	1.62^{a}	1.63^{a}	0.03	0.23
Abdominal fat (%BW)	2.40^{a}	1.86^{b}	1.67^{b}	1.55^{b}	2.02^{ab}	1.62^{b}	0.09	0.00

[a,b:] Means with the same superscript on the same line are not significantly different (P>0.05). P= probability. R_0=control ration without any supplement; R_{Th+Or}=R_0+0.01% mixture of EOs; R_{0C} = R_0 + 0.2% charcoal; R_{0C+Th} = R_0 + 0.2% charcoal + 0.01% Thyme EO; R_{0C+Or}= R_0 + 0.2% + 0.01% Oregano EO; $R_{0C+Th+Or}$= R_0 + 0.2% of charcoal + 0.01% mixture of EOs.

Table 4. Relative weight of digestion organs of broiler chickens as affected by thyme and oregano essential oils stabilized in *Canarium* seeds' charcoal at 49 days old

Digestive organs traits	**Treatments**							
	R_0	**R_{Th+Or}**	**R_{0C}**	**R_{0C+Th}**	**R_{0C+Or}**	**$R_{0C+Th+Or}$**	**SEM**	**P**
Gizzard (% BW)	1.59^{a}	1.57^{a}	1.48^{a}	1.60^{a}	1.62^{a}	$1,47^{a}$	0.03	0.42
Pancréas (% BW)	0.26^{a}	0.21^{ab}	0.20^{abc}	0.13^{c}	0.19^{abc}	$0,16^{bc}$	0.01	0.02
Intestin weight (g)	84.08^{a}	78.10^{bc}	80.30^{abc}	87.20^{ab}	77.90^{a}	74.20^{c}	1.75	0.02
Intestin length (cm)	210.08^{a}	204.60^{a}	204.50^{a}	204.70^{a}	202.10^{a}	205.20^{a}	2.71	0.96
Intestin density (g/cm)	0.43^{a}	0.38^{ab}	0.39^{ab}	0.43^{a}	0.39^{ab}	0.36^{b}	0.01	0.03

[a,b,c:] Means with the same superscript on the same line are not significantly different (P>0.05). P= probability. R_0=control ration without any supplement; R_{Th+Or}=R_0+0.01% mixture of EOs; R_{0C} = R_0 + 0.2% charcoal; R_{0C+Th} = R_0 + 0.2% charcoal + 0.01% Thyme EO; R_{0C+Or}= R_0 + 0.2% + 0.01% Oregano EO; $R_{0C+Th+Or}$= R_0 + 0.2% of charcoal + 0.01% mixture of EOs.

Tableau 5. Ileal and cæcal microbial load of 49 days old broiler chickens as affected by thyme and oregano essential oils stabilized in *Canarium* seeds' charcoal

Bacterial count	**Treatments**							
Log_{10} (10^7cfu/ml)	**R_0**	**R_{Th+Or}**	**R_{0C}**	**R_{0C+Th}**	**R_{0C+Or}**	**$R_{0C+Th+Or}$**	**SEM**	**P**
Ileum								
Lactic acid bacterial	8.48^{cA}	9.28^{bA}	9.30^{bC}	9.43^{bA}	9.73^{aA}	9.90^{aA}	0.09	0.00
E. coli	7.96^{cA}	8.64^{bB}	9.82^{aA}	8.88^{bB}	8.80^{bB}	8.55^{bcC}	0.15	0.00
Salmonella	7.72^{cA}	8.72^{bB}	9.58^{aB}	8.67^{bC}	9.71^{aA}	9.05^{bB}	0.15	0.00
SEM	0.19	0.10	0.07	0.11	0.15	0.19	--	--
p	0.300	0.011	0.000	0.000	0.000	0.000	--	--
Cæcum								
Lactic acid bacterial	8.13^{cA}	9.43^{abA}	9.52^{abA}	9.36^{bA}	9.20^{bA}	9.93^{aA}	0.13	0.00
E. coli	7.00^{dB}	7.81^{abC}	8.75^{aB}	7.54^{cC}	8.21^{bB}	8.54^{aB}	0.12	0.00
Salmonella	7.00^{dB}	8.37^{cB}	9.55^{aA}	8.76^{bB}	8.35^{cB}	8.19^{cC}	0.16	0.00
SEM	0.19	0.11	0.07	0.11	0.14	0.20	--	--
P	0.000	0.000	0.001	0.000	0.005	0.000	--	--

[a,b,c,d:] Means with the same superscript on the same line are not significantly different (P>0.05). P= probability. A.B: Means with the same superscript on the same column are not significantly different (P>0.05). R_0=control ration without any supplement; R_{Th+Or}=R_0+0.01% mixture of EOs; R_{0C} = R_0 + 0.2% charcoal; R_{0C+Th} = R_0 + 0.2% charcoal + 0.01% Thyme EO; R_{0C+Or}= R_0 + 0.2% + 0.01% Oregano EO; $R_{0C+Th+Or}$= R_0 + 0.2% of charcoal + 0.01% mixture of EOs.

Table 6. Serum biochemical parameters of 49 days old broiler chickens as affected by thyme and oregano essential oils stabilized in *Canarium* seeds' charcoal

Biochemical parameters	R_0	R_{Th+Or}	R_{0+C}	R_{0+Th}	R_{C0+Or}	$R_{C0+Th+Or}$	SEM	P
ASAT (U/L)	42,00^{ab}	26.25^{b}	38,06^{ab}	31,50^{b}	52,50^{a}	34,13^{b}	2.59	0.19
ALAT (U/L)	32,38^{a}	15.31^{bc}	30,28^{a}	8,17^{c}	10,21^{bc}	18,20^{b}	2.16	0.00
Creatinine (mg/dl)	2,63^{a}	2.02^{b}	1,67^{bc}	2,79^{a}	1,46^{c}	0,61^{d}	0.16	0.00
Urea (mg/dl)	7,09^{b}	7.06^{b}	7,34^{ab}	7,78^{a}	7,09^{b}	7,63^{ab}	0.09	0.06
Total cholesterol (mg/dl)	62,77^{bc}	81.82^{a}	89,08^{a}	76,20^{ab}	78,36^{a}	60,31^{c}	2.59	0.00
HDL-cholesterol (mg/dl)	30,94^{ab}	29.37^{ab}	23,18^{b}	27,51^{b}	43,71^{a}	19,41^{b}	2.25	0.02
LDL-cholesterol (mg/dl)	27,63^{ab}	19.29^{b}	11,52^{b}	17,40^{b}	38,69^{a}	11,02^{b}	1.70	0.00
Triglyceride (mg/dl)	13,70^{c}	19.09^{bc}	24,07^{b}	20,90^{b}	22,33^{b}	36,29^{a}	2.08	0.00
Total protein (g/dl)	2,26^{b}	2.34^{b}	2,47^{b}	2,40^{b}	2,94^{a}	2,96^{a}	0.07	0.00
Albumin (g/dl)	1.20^{c}	2.10^{cb}	2.83^{b}	3.8^{a}	3.93^{a}	2.14^{cb}	0.24	0.00
Globulin (g/dl)	0.80^{b}	1.48^{ab}	0.77^{b}	1.05^{b}	1.07^{b}	1.72^{a}	0.35	0.13

$^{a,b,c:}$ Means with the same superscript on the same line are not significantly different (P>0.05). P= probability. R_0=control ration without any supplement; R_{Th+Or}=R_0+0.01% mixture of EOs; R_{0C} = R_0 + 0.2% charcoal; R_{0C+Th} = R_0 + 0.2% charcoal + 0.01% Thyme EO; R_{0C+Or}= R_0 + 0.2% + 0.01% Oregano EO; $R_{0C+Th+Or}$= R_0 + 0.2% of charcoal + 0.01% mixture of EOs. ASAT: Aspartate aminotransferase, ALAT: Alanine aminotransferase

Table 7. Blood parameters of 49 days old broiler chickens as affected by thyme and oregano essential oils stabilized in *Canarium* seeds' charcoal

Blood parameters	Treatments							
	R_0	R_{Th+Or}	R_{0C}	R_{0C+Th}	R_{0C+Or}	$R_{0C+Th+Or}$	SEM	P
WBC (10^3/µl)	87.52	90.03	83.27	84.68	84.15	88.98	0.80	0.05
RBC (10^6/µl)	2.82	2.97	2.80	2.84	2.80	3.09	0.04	0.58
Hb (g/dl)	14.03	14.66	13.82	13.73	14.43	14.85	0.18	0.44
HCT (%)	33.98	36.28	33.15	33.00	34.48	36.55	0.73	0.67
MCV (fL)	120.18	122.21	118.48	116.27	122.98	118.40	1.04	0.42
MCH (pg)	49.80	49.36	49.48	48.35	52.68	48.07	0.63	0.36
PLT (10^3/µl)	27.83	46.83	38.17	29.83	27.83	38.25	2.71	0.23

WBC = White blood cell; RBC = red blood cell; Hb = Hemoglobin; HCT = Hematocrit; MCV = mean globular volume; MCH = Mean concentration of hemoglobin; PLT = Blood plate. R_0=control ration without any supplement; R_{Th+Or}=R_0+0.01% mixture of EOs; R_{0C} = R_0 + 0.2% charcoal; R_{0C+Th} = R_0 + 0.2% charcoal + 0.01% Thyme EO; R_{0C+Or}= R_0 + 0.2% + 0.01% Oregano EO; $R_{0C+Th+Or}$= R_0 + 0.2% of charcoal + 0.01% mixture of EOs.

DISCUSSION

In the present study, charcoal and EOs significantly (P < 0.05) enhanced feed intake in the finisher phase and throughout the experimental period as compared to the control diet without any supplement. Similar to this result, Kana et al. (2011) and Khattak et al. (2014) respectively reported that *Canarium charcoal* and blend of EOs stimulated feed intake, improved weight gain and offered health advantages to poultry. The mixture of EOs with charcoal resulted in a non significant increase in weight gain for about 6% compared to the control diet without charcoal and EOs. This result is in close agreement with Lee et al. (2003) and Jang et al. (2007) who reported no significant effect on the weight gain and live body weight of broilers fed on a commercial feed additive containing thymol and cinnamaldehyde. The upward trend noticed in weight gain in the present study could be due to the antibacterial activity of carvacrol and thymol, the main active compounds of oregano and thyme EOs which might modulate the gut microbiota by inhibiting pathogenic bacterial growth due to their selective antibacterial properties. A more balanced micriobiota population in gut could lead to a better efficiency in digestibility of nutrients, resulting in growth enhancement (Toghyani et al., 2010; Khattak et al., 2014). The weight gain improvement of broilers achieved in the present study could also be attributed to the positive effect of charcoal and EOs on nutrient digestibility through the stimulation of the digestive enzymes of the host as reported by Kana et al. (2011), Bento et al. (2013) and Parakesvakis et al. (2015) or by their antioxidant effects in the intestine (Karadas et al., 2014).

The mixture of EOs with charcoal induced a significant decrease ($P < 0.05$) in gain/feed ratio as compared to charcoal an individual's EO alone, suggesting a synergy between active compounds of oregano and thyme EOs. A number of *in vitro* studies also provided evidence of a synergy between EOs with different composition and origin (Malthlouthi et al., 2010; Bento et al., 2013). The enhancement of gain/feed ratio recorded in the present study suggested that the digestibility of feed and the absorption of the nutriments are better in chickens fed on diets supplemented with charcoal and charcoal enriched with EOs.

The feeding of broilers with charcoal enriched with thyme and oregano EOs resulted in a significant decrease in abdominal fat deposit. This low deposit of abdominal fat could be due to the effect of carvacrol and thymol present in these EOs on the metabolism of fat. Indeed, according to Zhang et al. (2007) the activation of the transient receptor (TRPV1) by active compounds like capsaicin found in some plants prevented the deposition of fat in mice and humans. It might be the case with carvacrol and thymol and other major compounds like linlool, p-cymene, α and γ-terpinene (Malthlouthi et al., 2010) found in thyme and oregano EOs used in the present study. The low fat deposit recorded here can explain the decrease in relative weight of the pancreas, suggesting the reduction in hormonal secretions by this organ with the enrichment of the charcoal by the EOs.

The intestine density (weight/length) which is an indication of villi size of the mucosa layer markedly decreased with the charcoal and EOs as compared to the control diet. This result agrees with the findings of Abdel-Fatter et al. (2008) and Kana et al. (2011) who respectively reported that the inclusion of organic acids and the charcoal from *Canarium schweinfurthii* seeds and maize cob in broilers diet tend to decrease the intestine density.

Irrespective to the bacteria species, bacteria count increased markedly with charcoal without EO and charcoal enriched with the EOs both in the ileum and cæcum compared to the control diet. Whatever the case, the numbers of lactic acid bacteria (beneficial bacteria) count were higher than *salmonella* and the *E. coli* (pathogens). This situation can explained the improvement in weight gain recorded in this study. The present result is in agreement with the findings of Lan et al. (2005) and Murry et al. (2006) who reported an increase in lactic acid bacteria population in the gut of healthy chickens. When the living conditions in the intestine is favorable, the lactic acid bacteria multiply and eliminate pathogenic bacteria (*Salmonella* and *Escherichia coli*) by acidifying the milieu and producing antibacterial substances like organic acids (Elaroussi et al., 2008). Although the proliferation of lactic acid bacteria stimulates the immune system of the chickens (Tiihonen et al., 2010; Amerah et al., 2011; Khattak et al., 2014), it was shown that EOs prevent the adhesion of the pathogens bacteria on their intestinal mucosa by stimulating the secretion of mucus (Jamroz et al., 2006).

This study revealed that feeding broilers with charcoal without EO and charcoal enriched with EOs resulted in an increase in serum content of cholesterol, triglyceride, total protein, albumin and globulin. The result of this study confirmed the findings of Kana et al. (2011) who reported the beneficial effects of dietary *Canarium* and maize cob charcoals on hematological and biochemical parameters in broilers. The increase in serum content of protein suggested the capacity of EOs to improve digestion and absorption of proteins as previously reported by Bento et al. (2013) and Krishan and Narang (2014) allowing a better use of protein in broiler chicken and thus an improvement of the weight gain. The decrease in HDL-cholesterol and LDL-cholesterol recorded in this study agrees with the results of Ali et al. (2007) who reported that the addition of thyme in the diet of chicken induce a significant decrease in the serum content in HDL-cholesterol and total cholesterol. The decrease in cholesterol content recorded in this study could be due to the inhibiting effects of thymol and carvacrol on HMG-CoA reductase, a key enzyme in cholesterol synthesis (Crowell, 1999). The enrichment of charcoal with thyme and oregano EOs and their mixture markedly decreased the serum content in ALAT. The present result contradicted the findings of Khattak et al. (2014) who recorded no significant effect on the serum content in ASAT and ALAT of broilers with the commercial product containing thymol, carvacrol, cinnamaldehyde, oregano, peppermint and pepper.

Blood parameters reflect the healthy state of an organism and any changes happening to it could be an indication of unbalance feeding or disease attack. This study revealed that feeding broilers with charcoal enriched with thyme and origano EOs did not have any significant effect on blood parameters. This finding is in close agreement with Kana et al. (2011) and Toghyani et al. (2010) who respectively reported that supplementing broiler chickens with *Canarium* and maize cob charcoals, and thyme powder did not have any marked effect on white and red blood cells counts, hemoglobin content and hematocrit percentage.

CONCLUSION

The result presented in the present study suggested that charcoal from *Canarium schweinfurthii* seeds can be used to entrap, stabilize and facilitate the incorporation of essential oils in poultry feed for gut microbiota modulation and for a better growth rate without any detrimental effect on serum and hematological parameters.

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Competing interests

The authors declare that they have no competing interests.

Author's contributions

Ngouana, Necdem, Kuiede and Yemdjie went to the field to carry out the research and collect the samples. Kana supervised the overall research work. Ngouana wrote the first draft before being revised by Kana, Meimandipour and Teguia, and approved by all the authors.

Consent to publish

All persons gave their informed consent prior to their inclusion in the study.

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