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Effects of Fresh *Cupressus sempervirens* Leaves Infusion on Growth Performance, Intestinal Microbiota and Haemato-Biochemical Parameters in Broilers

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Abstract

The accumulation of growth-promoting antibiotic residues in animal products and the resistance developed by bacteria in poultry farms has led to a search for natural compounds derived from plants. This study was designed to promote the production performance of broiler chickens using fresh *Cupressus sempervirens* leaves infusion. Fresh *Cupressus sempervirens* leaves were harvested, washed, chopped and ground to a paste using a blender and fermented for three days in a closed container at a rate of 500 g/L of water. The solution obtained was filtered and added at the rate of 2, 4, 6, 8 and 10 ml/L of drinking water. The chickens fed on the graded level of the solution were compared to a control ration without an additive and positive control group supplemented with 1 g antibiotic/kg feed. At the finisher phase and throughout the study period, water intake increased significantly ($P < 0.05$) with increasing levels of infusion. Feed intake decreased significantly ($P < 0.05$) with 2 and 4 ml of infusion/L drinking water. Live weight and weight gain were significantly ($P < 0.05$) higher with 6 ml/L, while feed conversion significantly ($P < 0.05$) decreased with the same treatment compared with the control treatment without additives (T0). Carcass characteristics were not significantly ($P > 0.05$) affected by the inclusion of *Cupressus sempervirens* infusion. Haematological parameters significantly ($P < 0.05$) increase independently of the rate of incorporation of the infusion into the drinking water, with the exception of RBCs, MCHT and PCT. Serum content in total protein,

globulins, LDL cholesterol and triglycerides were significantly ($P < 0.05$) high with 8 and 10 ml *Cupressus sempervirens* infusion/litre drinking water as compared to all other treatments. AST, ALT, urea, creatine, albumin, total cholesterol and HDL-cholesterol were not significantly affected. The lactic acid bacteria load increased significantly ($P < 0.05$) above 4 ml of infusion while *E. coli* and *salmonella* counts decreased significantly ($P < 0.05$) with infusion compared to the control without additive. In conclusion, 6 ml of *Cupressus sempervirens* infusion can be used as an alternative to antibiotic feed additives to promote growth performance in broilers.

Keywords

Broilers, *Cupressus sempervirens*, Growth Performance, Haemato-Biochemical Parameters, Intestinal Microbiota

1. Introduction

The livestock industry, and particularly the poultry industry, has been faced with a drop in productivity due to the ban on antibiotics as growth promoters in animal nutrition [1]. The use of antibiotics as growth promoters has led not only to the development of resistance in pathogenic microbes [2] [3], but also to health problems linked to the antibiotic residues in livestock products intended for human consumption [3]. This ban has led to a search for natural biodegradable compounds, derived from plants called phytobiotics that can produce the same effects as antibiotics with less or no impact on animal and human health and the environment [4]. Studies revealed the presence in phytobiotics of active compounds such as isoprenes, flavonoids, tannins and alkaloids that act on animal growth through their antimicrobial and antioxidant properties [5] [6]. A large number of studies involving phytobiotics have been carried out as feed additives. These include among others plant and spice [7] [8], essential oils [9] [10] and neem seed oil [4] [11].

Cupressus sempervirens is a tree that can grow up to 30 m tall, with leaves measuring 0.5 to 1 mm, dark green in colour and obtuse [12]. Native to the Mediterranean basin, it is widespread in central and northern Africa, Asia, southern Europe, North America and southern China [13]. In traditional medicine, the leaves of this tree are generally used to relieve pains such as colds, coughs, bronchitis, haemorrhoids, varicose veins, venous diseases and circulatory disorders [12]. Several studies have reported its antibacterial and antifungal [14], antiviral [15], antiparasitic and insecticidal [13] [16] antioxidant [17], anti-carcinogenic [18], hypolipidaemic [19], osteogenic [20] and antimicrobial [21] properties. All these properties are based on the presence of compounds such as alkaloids, flavonoids, tannins, saponins, phenols and several other biologically active compounds [22] that can act on animal growth [21]. In view of its properties and secondary metabolites, this plant could provide health and well-being,

which could positively affect animal growth performance. The general objective of this study was to evaluate the effect of incorporating an infusion of *Cupressus sempervirens* fresh leaves as a feed additive to mitigate the effects of antibiotics growth promoters in animal nutrition.

2. Material and Methods

2.1. Study Area

This study was carried out at the Teaching and Research Farm (TRF) of the University of Dschang, located at 05˚26' North latitude, 10˚26' East longitude and at an altitude of 1420 m. The prevailing climate is equatorial, characterized by two seasons. A rainy season lasts from March to November and a dry season covers the rest of the year. Rainfall varies between 1500 and 2000 mm per year. The average temperature is around 21˚C, the average annual insolation is 1837 hours and the average relative humidity is 76.8%.

2.2. Experimental Birds

A total of 336 day-old Cobb 500 chicks were used in this study for a period of 49 days. They were randomly assigned following a completely randomized design to 7 treatments replicated 3 times with 16 chicks each (8 males and 8 females). Water and feed were provided *ad libitum* to the birds. An anti-stress was given in drinking water during the first 3 days after the chicks' arrival. The latter was equally given before, during and after each handling and transfer of chicks from the brooder to the finishing house. An anti-coccidian [Vetacox®] and vitamins [AMINTOTAL®] were given through drinking water on 3 consecutive days each week, and from the fourth week onwards only anti-coccidian was given. The birds were vaccinated against infectious bronchitis [H120®] and Newcastle disease [Hitchner B1®] at seven days old and against Gumboro disease [IBA Gumboro®] at tenth day old. The vaccination recall was done on the eighteenth day.

2.3. Plant Material and Experimental Rations

Fresh leaves of *Cupressus sempervirens* were collected from a mature tree in full bloom in the city of Dschang, washed with water, chopped and then ground with a blender until they were reduced to a paste and soaked in a closed container of water for three days at a rate of 500 g/1liter. The resulting solution was filtered through Whatman paper (3 MM). The filtrate recovered represents the infusion. The antibiotic (Doxycycline) used in the positive control ration was purchased from a local veterinary pharmacy. Phytochemical analysis of the infusion was carried out by the chromatographic methods described by [23]. It revealed the presence of flavonoids, terpenoids, phenols, alkaloids, tannins, anthocyanins and anthraquinones and the absence of steroids and saponins (**Table 1**). The chicks were litter brooded at a density of 20 chicks/m^2 at the starter phase (1 - 21 days) and 10 chicks/m^2 at the finisher phase (22 - 49 days). A negative control ration (T0) without supplement (**Table 2**), and a positive control ration (R0+) containing

Table 1. Phytochemical composition of *Cupressus sempervirens* infusion.

Phytochemicals	Result
Alkaloids	+
Flavonoids	+
Phenols	+
Sterols	-
Tannins	+
Terpenoids	+
Saponins	-
Anthocyanins	+
Anthraquinones	+

Present: +; Absent: -.

Table 2. Composition of experimental rations.

Ingredients	Start	Finish
Maize	60	67
Remoulding	2	2
Cotton cake	5	5
Fish meal	5	5
Soya meal	22	15
Oeyster	1	1
CMAV 5%	5	5
Total	100	100
Analysed chemical composition		
Crude protein (%)	22.40	19.44
Crude cellulose (%MS)	4.00	4.35
Calculated chemical composition		
Metabolizable energy (Kcal/kg)	2956.10	3014.13
Crude protein (% DM/kg)	23.31	20.48
Energy/protein	126.82	147.16
Crude fibre (%)	5.25	5.38
Calcium (%)	1.17	1.15
Phosphorus (%)	0.48	0.46
Calcium/phosphorus	2.44	2.50
Lysine (%)	1.40	1.19
Methionine (%)	0.48	0.45
Lys/meth	2.92	2.50

CMAV 5% meat: Mineral, nitrogen and vitamin complex: Crude protein = 40%, Lysine = 3.3%, Methionine = 2.40%, Calcium = 8%, Phosphorus = 2.05%, Metabolisable energy = 2078 Kcal/kg.

1 g antibiotic (Doxycycline)/kg feed were formulated, and the other five treatments consisted of incorporating *Cupressus sempervirens* infusion at a rate of 2, 4, 6, 8 and 10 ml/L of drinking water and fed with the control ration (R0).

2.4. Growth Performance

Data were collected every 7 days on feed intake, live body weight, weight gain and feed conversion ratio. Feed was weighed and distributed to the birds daily and at the end of each week, the left overs were collected and weighed. Feed intake was calculated as the difference between the quantity served and the left over in each experimental unit. At the beginning of the trial and every 7 days thereafter, birds in each experimental unit were weighed and weekly weight gain was calculated as the difference between two consecutive weights. Feed conversion ratio (FCR) was calculated as the ratio of the amount of feed intake during the week and the weight gain of the same week.

2.5. Carcass Characteristics

At 49 days old, 10 chickens per treatment (05 males and 05 females) were fasted for 24 hours, weighed, sacrificed, plucked and eviscerated for carcass evaluation as proceeded by [24]. Carcass yield and relative weights of organs were respectively calculated by dividing the weights of carcass and that of organs by the live body weight of the bird.

2.6. Haematological and Serum Biochemical

From each slaughtered bird, blood was collected in 2 tests tubes, one containing anticoagulant (EDTA). Blood with anticoagulant was used for haematological analysis using a fully automatic blood cell counter (model PCE-210N Hong kong, China). Haematological parameters studied included White blood cell (WBC), Red blood cell (RBC), Haemoglobin (Hgb), Mean cell haemoglobin concentration (MCHC), Mean cell volume (MCV), Mean cell haemoglobin (MCH) and Packed cell volume (PCV). After centrifugation at 4000 rpm for 20 min of blood free from anticoagulant, serum was collected and preserved at −20˚C for the total protein, albumin, globulin, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), total cholesterol, high density lipoproteins (HDL), low density lipoprotein (LDL), triglycerides, urea and creatinine quantification, using the colorimetric method as prescribed by the non-specific chickens commercial kits (Spinreactkits)*.

2.7. Gut Microbiota

Before the chickens were sacrificed, faeces samples were aseptically collected using sterile swabs from the cloaca of 4 chickens per treatment and immediately transported to the laboratory for the identification and quantification of lactic acid bacteria, Salmonella and *Escherichia coli* respectively in the specific culture media as described by [25]. The culture medium used was respectively MRS AGAR, SS AGAR and Mac Conkey AGAR.

2.8. Statistical Analysis

All data collected were submitted to one-way analysis of variance (ANOVA). In case of a significant difference between treatments groups, Duncan's multiple range test was used to separate mean at the 5% significance level. The statistical software SPSS 20.0 (Statistical Package for Social Science) was used for the analyses.

3. Results

3.1. Growth Parameters

Table 3 summarises the overall growth performance of broilers supplemented with increasing levels of *Cupressus sempervirens* infusion through the drinking water. No significant difference ($P > 0.05$) was recorded between treatments at the starter phase, while at the finisher phase and over the whole trial period, water and feed intake were significantly higher with antibiotic supplemented diet compared to the control ration without additive and the other treatments. There was also a significant linear increase ($P < 0.05$) in water intake with increasing levels of *Cupressus sempervirens* infusion over the whole trial periods.

At the starter phase, the incorporation of *Cupressus sempervirens* filtrate into the drinking water induced no significant difference ($P > 0.05$) between treatments for live weight and weight gain. However, supplemented birds with 2 ml/L of this phytobiotic tend to decrease live weight and weight gain ($P > 0.05$). At the finisher phase and over the entire study period, chickens supplemented with the antibiotic and with 6 ml/L of *Cupressus sempervirens* filtrate in the drinking water recorded the highest live weight and weight gain ($P < 0.05$) compared to chickens fed on the control ration without additive and the other rations. From 6 ml/L inclusion of *Cupressus sempervirens*, live weight and weight gain decreased linearly with increasing levels of incorporation.

At the starter phase, analysis of variance revealed no significant effect ($P > 0.05$) of infusion on feed conversion ratio while at the finisher phase and over the entire duration of the trial, chickens supplemented with the antibiotic and 6 ml/L of *Cupressus sempervirens* infusion recorded the lowest feed conversion ratio ($P < 0.05$).

3.2. Carcass Characteristics

Carcass characteristics expressed as % live weight of chickens supplemented with increasing levels of *Cupressus sempervirens* infusion trough drinking water are presented in Table 4. It can be seen that the infusion of fresh *Cupressus sempervirens* leaves had no significant effect ($P > 0.05$) on carcass characteristics and relative weights of organs compared to carcass characteristics of chickens fed on control rations.

3.3. Haematological Parameters

The summary of the effect of the increasing rate of *Cupressus sempervirens* infusion in drinking water on the haematological parameters of broilers is

Table 3. Variation of broiler's growth performance with respect to in-water incorporation level of *Cupressus sempervirens* infusion.

Period (days)	Control rations		Incorporation levels (ml/liter of drinking water)					P
	T0	T0+	2	4	6	8	10	
Water intake (ml)								
01 to 21	2634.65 ± 146.86	2702.50 ± 109.77	2603.71 ± 189.46	2591.22 ± 165.59	2690.79 ± 77.60	2672.19 ± 175.70	2672.27 ± 94.19	0.947
22 to 49	8805.04 ± 109.51^{d}	9823.62 ± 139.38^{a}	8701.88 ± 187.89^{d}	9024.03 ± 194.04^{cd}	9223.47 ± 289.27^{bc}	9300.66 ± 49.03^{bc}	9443.18 ± 171.31^{b}	0.000
01 to 49	1439.69 ± 253.18^{cd}	12526.12 ± 217.10^{a}	11305.59 ± 155.30^{d}	11615.25 ± 353.31^{bcd}	11914.26 ± 349.57^{bc}	11972.85 ± 183.37^{b}	12115.45 ± 244.54^{ab}	0.001
Feed intake (g)								
01 to 21	1033.32 ± 62.19	1018.56 ± 27.54	1024.75 ± 15.00	1007.31 ± 19.95	1042.74 ± 43.21	1009.29 ± 15.65	1023.83 ± 24.26	0.888
22 to 49	4090.62 ± 87.92^{cd}	4426.47 ± 103.38^{a}	3927.83 ± 60.53^{d}	4063.46 ± 121.08^{cd}	4379.57 ± 126.79^{a}	4306.90 ± 98.41^{ab}	4180.33 ± 112.21^{bc}	0.000
01 to 49	5123.94 ± 127.61^{bcd}	5445.03 ± 82.41^{a}	4952.58 ± 68.89^{d}	5070.77 ± 104.03^{cd}	5422.31 ± 162.88^{a}	5316.19 ± 90.13^{ab}	5204.16 ± 133.67^{bc}	0.001
Live body weight (g)								
01 to 21	733.31 ± 21.44	766.58 ± 27.55	711.88 ± 49.45	724.92 ± 29.43	778.45 ± 42.67	733.56 ± 27.31	732.15 ± 10.28	0.230
22 to 49	2429.82 ± 88.94^{bc}	2713.33 ± 50.91^{a}	2326.85 ± 19.82^{c}	2401.03 ± 66.88^{bc}	2625.39 ± 86.35^{a}	2489.89 ± 70.38^{b}	2447.25 ± 95.00^{bc}	0.000
Weight gain (g)								
01 to 21	689.13 ± 21.44	722.40 ± 27.55	667.70 ± 49.45	680.74 ± 29.43	734.27 ± 42.67	689.38 ± 27.31	687.97 ± 10.28	0.230
22 to 49	1696.51 ± 81.56^{cd}	1946.75 ± 47.93^{a}	1614.98 ± 61.41^{d}	1676.11 ± 39.96^{cd}	1846.94 ± 91.15^{ab}	1756.33 ± 43.92^{bc}	1715.11 ± 95.68^{cd}	0.001
01 to 49	2385.64 ± 88.94^{bc}	2669.15 ± 50.91^{a}	2282.68 ± 19.82^{c}	2356.85 ± 66.88^{bc}	2581.21 ± 86.35^{a}	2445.71 ± 70.38^{b}	2403.08 ± 95.00^{bc}	0.000

Continued

	Feed conversion ratio							
01 to 21	1.50 ± 0.11	1.41 ± 0.03	1.54 ± 0.12	1.48 ± 0.08	1.42 ± 0.02	1.47 ± 0.08	1.49 ± 0.5	0.527
22 to 49	2.41 ± 0.08[a]	2.27 ± 0.01[b]	2.43 ± 0.05[a]	2.42 ± 0.02[a]	2.37 ± 0.12[ab]	2.45 ± 0.04[a]	2.44 ± 0.10[a]	0.019
01 to 49	2.15 ± 0.03[a]	2.04 ± 0.02[b]	2.17 ± 0.02[a]	2.15 ± 0.02[a]	2.10 ± 0.07[ab]	2.17 ± 0.05[a]	2.17 ± 0.06[a]	0.020

[a,b,c,d]means bearing the same letter on the same line are not significantly different (P > 0.05). **T0:** control ration with no additives; **T0+**: ration with no additive control + 1 g/L doxycycline; **P**: P-value.

Table 4. Variation in relative organ weight and carcass yield as a function of *Cupressus sempervirens* infusion rate.

Characteristics (%LBW)	Control rations		Incorporation levels (ml/liter of drinking water)					P
	T0	T0+	2	4	6	8	10	
Carcass yield (%)	74.31 ± 0.96	74.91 ± 1.38	73.47 ± 2.70	74.84 ± 1.78	75.08 ± 1.09	74.78 ± 0.80	74.53 ± 1.89	0.370
Head	2.18 ± 0.17	2.08 ± 0.19	2.19 ± 0.25	2.11 ± 0.19	2.17 ± 0.34	2.15 ± 0.26	2.11 ± 0.17	0.875
Legs	3.69 ± 0.44	3.70 ± 0.48	3.82 ± 0.51	3.72 ± 0.43	3.58 ± 0.38	3.63 ± 0.30	3.55 ± 0.60	0.934
Liver	1.61 ± 0.19	1.63 ± 0.15	1.63 ± 0.14	1.73 ± 0.19	1.61 ± 0.10	1.66 ± 0.15	1.71 ± 0.21	0.602
Heart	0.51 ± 0.07	0.46 ± 0.11	0.48 ± 0.08	0.50 ± 0.05	0.47 ± 0.07	0.45 ± 0.09	0.52 ± 0.11	0.561
Pancreas	0.23 ± 0.04	0.21 ± 0.06	0.20 ± 0.08	0.23 ± 0.06	0.23 ± 0.04	0.19 ± 0.05	0.23 ± 0.06	0.579
Abdominal fat	1.80 ± 0.39	1.78 ± 0.54	1.76 ± 0.64	1.31 ± 0.59	1.67 ± 0.47	1.52 ± 0.41	1.81 ± 0.12	0.213

T0: control ration with no additives; **T0+**: ration with no additive control + 1 g/L doxycycline, BW: Body Weight; **P**: P-value.

presented in Table 5. The analysis of variance shows that Haematological parameters were significantly ($P < 0.05$) increase independently of the rate of incorporation of the infusion, with the exception of red blood cells, mean corpuscular haemoglobin content and platelets. Chickens fed with 6 ml of infusion per litre of drinking water had a significantly ($P < 0.05$) lower white blood cell count compared to chickens in the control group. Furthermore, chickens fed with 4 and 10 ml of infusion per litre of drinking water respectively recorded significantly ($P < 0.05$) higher mean corpuscular haemoglobin concentration (MCHC) and a blood platelet concentration.

Table 5. Effects of *Cupressus sempervirens* infusion rate on haematological parameters in broilers.

Paramètres	Control rations		Incorporation levels (ml/liter of drinking water)					P
	T0	T0+	2	4	6	8	10	
WBC (10^9/L)	314.29 ± 32.24^{a}	259.60 ± 9.34^{b}	213.85 ± 33.35^{c}	247.73 ± 32.14^{b}	207.93 ± 11.24^{c}	210.57 ± 34.14^{c}	210.03 ± 24.54^{bc}	0.000
RBC (10^{12}/L)	4.19 ± 0.94	5.13 ± 0.24	5.07 ± 1.28	5.91 ± 0.27	5.84 ± 0.16	4.32 ± 1.65	4.17 ± 1.53	0.215
HGB (g/dL)	23.18 ± 2.27^{c}	24.45 ± 0.92^{bc}	26.26 ± 2.53^{abc}	29.13 ± 0.64^{a}	28.53 ± 0.78^{ab}	22.77 ± 5.34^{bc}	25.38 ± 3.65^{ab}	0.021
HCT (%)	71.90 ± 1.20^{d}	73.70 ± 1.80^{cd}	75.39 ± 2.82^{c}	84.38 ± 1.10^{a}	81.63 ± 1.71^{b}	75.20 ± 3.14^{c}	82.17 ± 3.18^{ab}	0.000
MCV (%)	144.18 ± 2.93^{a}	145.00 ± 2.39^{a}	137.75 ± 3.82^{b}	138.67 ± 1.50^{b}	139.87 ± 0.95^{ab}	144.10 ± 2.30^{a}	143.83 ± 2.12^{a}	0.004
MCH (pg)	49.30 ± 3.17	47.65 ± 0.76	49.68 ± 3.73	49.27 ± 1.29	48.83 ± 0.25	48.33 ± 2.02	49.20 ± 1.66	0.275
MCCH (g/dL)	33.30 ± 1.09^{c}	33.13 ± 0.46^{c}	34.98 ± 0.43^{ab}	35.53 ± 1.25^{a}	34.90 ± 0.35^{ab}	33.93 ± 0.98^{bc}	34.20 ± 1.08^{ab}	0.000
PLT (10^9/L)	72.00 ± 5.76^{b}	42.25 ± 7.91^{d}	59.25 ± 4.79^{c}	70.67 ± 2.52^{b}	70.00 ± 7.00^{bc}	67.67 ± 5.86^{bc}	113.67 ± 7.37^{a}	0.000
PCT (%)	0.03 ± 0.01	0.05 ± 0.04	0.04 ± 0.02	0.10 ± 0.08	0.06 ± 0.03	0.27 ± 0.38	0.06 ± 0.03	0.412

^{a,b,c,d}means bearing the same letter on the same line are not significantly different ($P > 0.05$). **T0:** control ration with no additives; **T0+**: ration with no additive control + 1 g/L doxycycline; P = Probability; WBC = White Blood Cells; RBC = Red Blood Cells; Hgb = Haemoglobin; HCT = Haematocrit; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; MCC = Mean Corpuscular haemoglobin Concentration; PCV = Packed Cell Volume; **P**: P-value.

3.4. Biochemical Parameters

Table 6 presents the effects of increasing levels of *Cupressus sempervirens* infusion on the biochemical parameters of broilers. Markers of hepatic functions (ALAT and ASAT) and kedneys functions (urea and creatinine) were not significantly ($P > 0.05$) affected by *Cupressus sempervirens* infusion whatever the rate in drinking water. Feeding broilers with *Cupressus sempervirens* infusion significantly ($P < 0.05$) increased total protein and globulin content in a linear manner with increasing rate of the infusion.

3.5. Intestinal Microbiota

The results of the bacterial colonies on the intestinal microbiota are summarised in Table 7. Broilers fed on the ration without the supplement had a significantly ($P < 0.05$) lower lactic acid bacterial load compared to all other treatments groups. The increasing rates of *Cupressus sempervirens* infusion into the drinking water resulted in a significant ($P < 0.05$) reduction of *Escherichia coli* count compared to the control ration without additive.

4. Discussion

The inclusion of large quantities of feed additives is likely to considerably reduce the feed intake in animals [26] [27]. In this study, the reduction in feed intake in broilers fed on rations supplemented with 8 and 10 ml *Cupressus sempervirens filtrate/L drinking* water could be related to the bitterness of the water induced by the presence of alkaloids in the high levels of the infusion. This result is in line with previous research by [8] who reported a significant drop in feed intake with the incorporation of 5 g of *Pentadiplandra brazzeana* powder/L of drinking water. These authors explain these results by the presence of alkaloids in the spice, which progressively induced a bitter taste in the drinking water that was not appreciated by the chickens, causing a drop in water intake and consequently in feed intake. It was also noted in this study that the feed intake of chickens fed on 6 ml/L of *Cupressus sempervirens* infusion was comparable to that of the chickens given the antibiotic, which was significantly higher than that of the control group. This result contradicts the finding of [28] who repported a significant increase in feed intake with the increasing levels of methenamine in drinking water of broilers.

Live weight and weight gain at the finisher phase and over the entire study period of broilers supplemented with antibiotic and 6 ml of *Cupressus sempervirens* filtrate were significantly high as compared to all other treatments groups. These results are in line with the findings of [29] who reported that 50 ml of ginger extract per litre of drinking water significantly improved the final live weight and weekly weight gain of broilers; and the results of [30] who reported a significantly positive effect on the weight gain of rats fed with *Cupressus sempervirens* extaits. This beneficial effect of live weight and weight gain with this phytobiotic in drinking water was explained by the presence of phenolic, terpenoic, and alkaloid compounds that are also present in *Cupressus sempervirens*.

Table 6. Effects of increasing rate of *Cupressus sempervirens* infusion in water on biochemical parameters of broilers.

Parameters	Control rations		Incorporation levels (ml/liter of drinking water)					P
	T0	T0+	2	4	6	8	10	
ALAT (U/I)	9.38 ± 24.22	123.80 ± 66.88	79.92 ± 56.81	124.69 ± 48.17	80.35 ± 51.40	99.60 ± 66.12	147.66 ± 68.12	0.515
ASAT (U/I)	139.71 ± 56.98	93.00 ± 57.74	103.83 ± 47.20	99.31 ± 73.98	112.88 ± 20.11	137.81 ± 39.13	90.23 ± 51.78	0.439
Urea (mg/dl)	10.89 ± 8.23	13.14 ± 8.70	22.67 ± 14.21	19.25 ± 6.23	10.61 ± 7.77	27.00 ± 23.03	18.42 ± 7.65	0.258
Creatinine (mg/dl)	0.43 ± 0.13	0.63 ± 0.44	0.89 ± 0.92	0.59 ± 0.33	0.41 ± 0.23	0.59 ± 0.37	0.51 ± 0.20	0.281
Total protein (mg/dl)	2.65 ± 1.16^{d}	6.05 ± 1.45^{ab}	3.24 ± 2.12^{cd}	4.56 ± 2.23^{c}	5.01 ± 1.59^{bc}	7.37 ± 1.69^{a}	7.58 ± 1.41^{a}	0.000
Albumin (mg/dl)	3.03 ± 1.21	3.11 ± 0.71	2.84 ± 0.63	3.06 ± 1.06	2.50 ± 0.73	3.49 ± 1.04	3.61 ± 1.46	0.182
Globulin (mg/dl)	0.73 ± 0.63^{c}	2.94 ± 1.91^{ab}	1.91 ± 0.78^{bc}	2.44 ± 0.61^{bc}	2.52 ± 1.11^{bc}	3.88 ± 1,35$^{\cdot}$	3.97 ± 1.42^{a}	0.000
Albumin/ globulin (mg/dl)	2.60 ± 0.98	1.09 ± 0.47	1.62 ± 0.52	1.34 ± 0.62	1.37 ± 1.07	1.04 ± 0.66	1.12 ± 0.90	0.696
Total cholesterol (mg/dl)	106.56 ± 42.36	141.37 ± 24.15	132.39 ± 82.53	135.43 ± 36.66	124.92 ± 35.21	172.87 ± 49.04	138.66 ± 60.30	0.093
HDL cholesterol (mg/dl)	35.76 ± 7.30	33.01 ± 10.95	29.02 ± 15.64	37.88 ± 8.88	32.47 ± 7.13	28.94 ± 4.69	31.65 ± 9.76	0.474
LDL cholesterol (mg/dl)	60.35 ± 36.35^{b}	86.73 ± 24.92^{ab}	68.68 ± 80.15^{ab}	80.54 ± 41.32^{ab}	69.89 ± 36.81^{ab}	111.11 ± 53.40^{a}	84.50 ± 62.57^{ab}	0.019
Triglyceride (mg/dl)	52.20 ± 7.18^{c}	108.15 ± 50.11^{ab}	106.17 ± 29.33^{ab}	85.02 ± 16.20^{bc}	112.81 ± 31.59^{ab}	126.57 ± 33.79^{a}	112.56 ± 37.72^{ab}	0.003

^{a,b,c,d}means bearing the same letter on the same line are not significantly different ($P > 0.05$). **T0:** control ration with no additives; **T0+**: ration with no additive control + 1 g/L doxycycline; **P**: P-value.

Table 7. Variation of broilers intestinal microbial count as affected by the infusion of fresh leaves of *Cupressus sempervirens* rate in drinking water.

Bacterial load log10 (CFU)	Control rations		Incorporation levels (ml/liter of drinking water)					P
	T0	T0+	2	4	6	8	10	
Lactic acid bacteria	6.80 ± 0.16^{c}	8.78 ± 0.08^{a}	7.72 ± 0.16^{b}	8.78 ± 0.30^{a}	8.94 ± 0.12^{a}	8.77 ± 0.16^{a}	9.02 ± 0.17^{a}	0.000
Escherichia coli	8.02 ± 0.10^{a}	7.02 ± 0.35^{bc}	7.37 ± 0.07^{b}	7.12 ± 0.07^{bc}	7.40 ± 0.04^{b}	6.99 ± 0.36^{bc}	6.86 ± 0.24^{c}	0.000
Salmonella	8.91 ± 0.18^{ab}	8.59 ± 0.06^{cd}	8.84 ± 0.03^{abc}	8.68 ± 0.02^{bc}	8.90 ± 0.26^{ab}	8.97 ± 0.03^{a}	8.35 ± 0.18^{d}	0.001

^{a,b,c,d}means bearing the same letter on the same line are not significantly different ($P > 0.05$). **T0:** control ration with no additives; **T0+**: ration with no additive control + 1 g/L doxycycline; **P**: P-value.

These active compounds can reduce host microbial competition for nutrients by reducing pathogenic bacteria. The improvement in live weight and weight gain in broilers fed on antibiotic supplemented feed could be explained by its ability to modulate the microbiota by reducing pathogenic bacteria (*Escherichia coli* and Salmonella).

The feed conversion ratio (FCR) at the finisher phase and over the entire trial period was significantly higher than that of chickens supplemented with antibiotics. This result is in agreement with the work of [28] who reported that the feed conversion ratio of broilers on the additive-free ration was significantly higher than that of the positive control batch, but remained comparable to rations fed methenamine in the drinking water. These results contradict those of [7] and [31] who reported a decrease in feed conversion in broilers and quail with the inclusion of 0.2%, 0.4%, and 0.6% of *Dichostachys glomerata* powder in the feed.

Increasing rates of *Cupressus sempervirens* infusion in broiler drinking water, had no significant effect on carcass characteristics and relative weights of organs. This result is in agreement with the results of [32], who recorded no significant effect on carcass yield, gut weight, the relative weight of liver, gizzard, the pancreas of broilers fed Livol (1 ml/2litre water), Livotal (1 ml/4litre water) and hepato promotor (1 ml/4litre water) which are commercial phytobiotics. Similarly, [28] reported that the increasing levels of methenamine in the drinking water of broilers had no significant effect on carcass yield and relative organ weights compared with chickens from control batches. In the same line, [8] found that the inclusion of increasing rates of *Pentadiplandra brazzeana* powder in the drinking water of broilers significantly affected carcass yield, but not digestive organs. Increasing levels of *Cupressus sempervirens* filtrate in the drink-

ing water had no significant effect on abdominal fat. This result corroborates those of [31] who reported that supplementing Japanese quails with gradual levels of *Dichostachys glomerata* fruit powder indicated no significant effect on relative abdominal fat weight. In contrast, [33] reported that the incorporation of 1.5 g/kg of *Capsicum annum into the broiler* ration resulted in a 43.4% reduction in abdominal fat compared with the control ration.

Blood parameters reflect the health status of an organism and any changes in these could indicate a dietary imbalance or microbial attack [8] [34] and animals with good blood composition are likely to perform well [35]. In the present study, red blood cell count, mean corpuscular haemoglobin content and platelet counts were not significantly affected by the phytobiotic. The white blood cells of the broilers in the control group without the additive were significantly higher than those of broilers supplemented with antibiotic, while the Hgb, VGM, CCMH and PLT of the chickens in the control ration were significantly lower with *Cupressus sempervirens* infusion. This is different from the finding of [29] which revealed an increase in red blood cell count in broilers receiving ginger extracts, and that of [36] which showed no significant effect on white blood cell count when 0.25% garlic, ginger and the garlic-ginger mixture were incorporated into the broiler feed. The present result corroborates that of [37] who stated that in broilers the haemoglobin level increased with aqueous garlic extracts (20 ml/L) in the drinking water. The present result is different from the findings of [38] who recorded no significant effects on haemoglobin levels in chickens fed different rates of garlic.

Supplementing broilers with increasing levels of *Cupressus sempervirens* had no significant effect on ASAT and ALAT concentrations. This result suggests that the active principles present in this plant have no harmful effect on the liver. This result corroborates the work of [39] who reported that ginger and garlic essential oils administered by gavage at doses of 10, 20 and 40 mg/kg/day had no effect on serum transaminase activity (ALAT and ASAT). This observation contradicts the results of [40] who reported that the addition of 5% ginger, garlic and cinnamon to the turkey ration reduced the concentration of ALAT and ASAT. Serum urea is an index that reflects the status of protein metabolism, renal function and body nutrition. Serum urea and creatinine concentrations were not significantly affected by increasing levels of *Cupressus sempervirens* in drinking water. This result is in agreement with that of [36] who reported that the addition of 0.25% ginger, garlic and the ginger-garlic mixture to the broiler ration did not significantly affect creatinine and urea concentrations. Feeding chickens with increasing levels of infusion of this plant through the drinking water did not significantly affect the serum cholesterol profile. This result contradicts the work of [19] who reported that the addition of thyme to the broilers' ration induced a significant drop in serum levels of HDL, total cholesterol and total lipids.

The increasing levels of *Cupressus sempervirens* in the broiler drinking water induced a significant increase in the Lactobacilli count compared to the negative

control ration. The number of *Escherichia coli* decreased significantly with the increasing levels of *Cupressus sempervirens* filtrate in drinking water compared to the negative control ration, while the Salmonella load of the control ration was comparable with the other treatments. [41] reported that when conditions in the intestinal environment become favourable, lactic acid bacteria multiply and selectively eliminate pathogenic bacteria (Salmonella and *Escherichia coli*) due to their acidifying properties and their ability to produce antibacterial substances such as organic acids. The hydrophobic compounds present in plants that penetrate the bacterial cell membrane disintegrate the membrane structure and cause leaks, making the microbes less virulent. Contrary to the present result, [42] reported a significant increase in the number of Salmonella, unlike the number of *Escherichia coli*, which was not affected by the supplementation of silver nanoparticles in the drinking water of broilers. Birds supplemented with 10 ml of *Cupressus sempervirens* recorded the lowest Salmonella and *Escherichia coli* loads. This suggests that, at this dose, the concentration of active compounds present in this phytobiotic has a positive effect on the inhibition of pathogenic bacteria.

5. Conclusion

Incorporating 6 ml of *Cupressus sempervirens* infusion per litre of drinking water can be used as a substitute for antibiotics to modulate the intestinal microbiota and improve nutrient absorption, with a positive impact on the growth performance of broiler chickens, without any toxic effect on vital organs such as the liver and kidneys.

Conflicts of Interest

The authors declare no conflict of interest.

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