

## Growth performances and serum biochemical response of broiler chickens fed on diet supplemented with *Tetrapleura tetraptera* fruit powder as substitute to antibiotic growth promoters

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**ABSTRACT:** Due to the development of microbial resistance to antibiotics and their potential side effects in human, there is a growing interest in plant feed additives in livestock production. This study was designed to assess the effects of dietary inclusion of *Tetrapleura tetraptera* fruit powder on growth performances and serological profile of broiler chickens. A total of 192 day old Cobb 500 chicks were randomly assigned to 4 treatments of 48 chicks each. Negative control group (R0-) was fed basal diet without supplement, positive control group (R0<sup>+</sup>) was fed on control diet supplemented with 0.1% antibiotic, groups 3 and 4 were fed on control diet supplemented with 0.2% (0.2T) and 0.4% (0.4T) *T. tetraptera* fruit powder respectively. Throughout the study period, 0.2% *T. tetraptera* markedly ( $P<0.05$ ) increased live body weight as compared to 0.4% and negative control diet. The lowest feed conversion ratio was recorded on chickens fed on antibiotic (2.02) and 0.2% *T. tetraptera* (2.03). Carcass yield significantly decreased ( $P<0.05$ ) with 0.4% *T. tetraptera* fruit powder. The lowest cost of production was recorded with the lowest level (0.2%) of this phytobiotic. 5. Irrespective of the incorporation level, this phytobiotic decreased serum content of creatinine, alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) and increased serum concentration of urea compared to the negative control diet. *T. tetraptera* powder at 0.2% could be used as feed additive to replace antibiotic growth promoter for a better growth performances and to produce antibiotics residues free chicken meat.

**KEYWORDS:** Antibiotic resistance, broiler chickens, feed additive, phytobiotic, *Tetrapleura tetraptera*.

### 1 INTRODUCTION

There is growing interest in herbal feed additive (phytobiotic) in livestock production due to development of microbial resistance to antibiotic feed additives [1]. Phytobiotic is any compound of vegetable origin which is incorporated in animal feed to improved growth performances through its anti-oxidative and antimicrobial action, palatability improvement and gut equilibrium maintenance [2], [3], [4], [5]. Under the term phytobiotic are gathered the herbs, spices as well as essential oils and extracts of plants [6]. Active properties of phytobiotics are composed of many molecules [7], [8] having different activities, including antibacterial properties [9], antioxidant activities [10], anti-inflammatory and immunomodulatory properties [8], [11].

Certain phytobiotics improve the activity of the digestive enzymes and the absorption of nutrients [12]. Kongmun *et al.* [13] reported that the use of garlic (*Allium sativum*) as feed additive, improved the digestibility of nutrients, growth and carcass characteristics of broiler. Uchechi *et al.* [14] reported that the fruit of *Tetrapleura tetraptera* known in Cameroun as "quatre cotés" possesses antibacterial compounds such as saponin, flavonoids, phenols, alkaloids, tannins. In addition, Jane *et al.* [15] reported that *T. tetraptera* fruit has antioxidant properties. Despite antibacterial, antioxidant and hepatoprotector

properties no study on its effects on animal production performances has been reported. The main objective of the present study is to evaluate the effects of dietary supplementation of *Tetrapleura tetraptera* fruit powder in order to mitigate the problems involved in antibiotic growth promoter in poultry industry.

## 2 MATERIALS AND METHODS

### 2.1 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 to 2000 mm per annum over a 9 months rainy season (March to November).

### 2.2 BIRDS, DIETARY TREATMENTS AND EXPERIMENTAL DESIGN

*Tetrapleura tetraptera* fruits were bought at the local market then ground in a Harmed mill, sieved and used as feed additives in broiler's feed. Antibiotics used (Doxycycline®) in positive control diet were bought from a local veterinary pharmacy. Three experimental diets were formulated from a negative control ratio (R0-) (Table 1) by incorporating 0.1% of antibiotic (R0+), 0.2% (0.2T) and 0.4% (0.4T) of *T. tetraptera* fruit powder.

**Table 1: Proximate nutrients composition and price of experimental diets**

Ingredients (%)	Starter	Finisher
Maize	59	60
Wheat grain	3	8
Soybean Meal 49	23	13
Coton meal	5	6
Fish meal	6	5.5
Borne meal	0.5	0.5
Osher shell meal	0.5	0.5
Palm oil	1	1.5
Premix 5%*	5	5
Total	100	100
Calculated proximate nutrients composition		
Metabolizable Energy (kcal/kg)	2961.71	3005.80
Crude Protein (%)	23.32	20.54
Energy/protein ratio	126.98	146.37
Lysine (%)	1.4	1.20
Methionine (%)	0.48	0.45
Calcium (%)	1.11	1.32
Phosphore (%)	0.54	0.58
Crude fibre (%)	4.76	4.91
Price (francs CFA/ kg)	311	283.75

Premix 5%: crude proteins=40%, Lys=3.3%, Meth=2.40%, Ca=8%, P=2.05%, metabolizable energy=2078kcal/kg

A total of 192 day old Cobb 500 strain broiler chicks were randomly assigned to four treatment diets including negative control diet in a completely randomized design with 48 birds per treatment. Each group was further sub divided into 4 replicates of 12 birds each (06 males and 06 females). 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*.

### **2.3 GROWTH AND CARCASS TRAITS**

Data on feed intake, body weight gain were collected and used to calculate feed conversion ratio and carcass characteristics parameters. At the end of the feeding trial (49 days) 10 birds (5 males and 5 females) from each treatment group were randomly selected, fasted for 24 hours and slaughtered for carcass evaluation. Blood from each slaughtered bird was collected for biochemical analysis.

### **2.4 SERUM BIOCHEMICAL ANALYSIS**

From each slaughtered chicken, blood was collected in test tube and serum obtained after centrifugation was preserved at -20°C for the evaluation of biochemical parameters. Biochemical parameters consisted of total protein, albumin, globulin, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), total cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride, urea and creatinin using colorimetric method as prescribed by the Chronolab® commercial kits (Barcelona, Spain).

### **2.5 COST OF PRODUCTION**

The cost of a kg of feed was calculated based on the price of each ingredient as practiced in the local market. The cost of feed intake was obtained by multiplying the average feed intake by the price of a kg of the corresponding diet. The cost of production of a kilogram of live body weight was calculated by multiplying the cost of the kg of feed by the corresponding feed conversion ratio.

### **2.6 STATISTICAL ANALYSIS**

The data were analyzed using Analysis of Variance of the Statistical Package for Social Science (SPSS 21.0) software. Duncan's multiple range tests was used for multiple comparisons. Probability values less than 0.05 were considered as significant [17].

## **3 RESULTS**

### **3.1 PERFORMANCES AND CARCASS TRAITS**

Table 2 summarizes the effects of *T. tetraptera* fruit powder on feed intake (FI), live body weight (BW), body weight gain (BWG) and the feed conversion ratio (FCR) of broiler chickens. All the studied parameters were significantly affected ( $P < 0.05$ ) by the treatments. Throughout the production period (1 to 49 days), the lowest FI was recorded with the ration supplemented with *T. tetraptera* fruit powder as compared to negative and positive control diets. During brooding period (22 to 49 days), the lowest FI ( $P > 0.05$ ) was recorded with ration supplemented with 0.2% of this phytobiotic.

**Table 2: Effects of *T. tetraptera* on growth performances of broiler chickens**

Study phases (days)	Rations				SEM	P
	RO-	RO+	0.2T	0.4T		
Feed intake (g)						
1 - 21	1003.29 <sup>ab</sup>	1024.38 <sup>a</sup>	992.96 <sup>b</sup>	965.542 <sup>c</sup>	25.26	0.001
22 - 49	4746.38 <sup>ab</sup>	4825.37 <sup>a</sup>	4483.54 <sup>c</sup>	4595.4 <sup>bc</sup>	182.59	0.002
1 - 49	5749.67 <sup>a</sup>	5849.74 <sup>a</sup>	5476.5 <sup>b</sup>	5560.94 <sup>b</sup>	196.13	0.001
Live body weight (g)						
1 - 21	763.917 <sup>a</sup>	774.604 <sup>a</sup>	717.104 <sup>b</sup>	618.979 <sup>c</sup>	66.09	0.000
1 - 49	2584 <sup>c</sup>	2929.83 <sup>a</sup>	2730.53 <sup>b</sup>	2069.74 <sup>d</sup>	431.16	0.000
Body weight gain (g)						
1 - 21	726.957 <sup>a</sup>	737.644 <sup>a</sup>	680.14 <sup>b</sup>	582.019 <sup>c</sup>	17.59	0.000
22 - 49	1820.08 <sup>c</sup>	2155.22 <sup>a</sup>	2013.42 <sup>b</sup>	1450.76 <sup>d</sup>	94.10	0.000
1 - 49	2547.04 <sup>c</sup>	2892.87 <sup>a</sup>	2693.57 <sup>b</sup>	2032.78 <sup>d</sup>	434.26	0.000
Feed conversion ratio						
1 - 21	1.38 <sup>c</sup>	1.39 <sup>c</sup>	1.46 <sup>b</sup>	1.65 <sup>a</sup>	0.12	0.000
22 - 49	2.62 <sup>b</sup>	2.24 <sup>c</sup>	2.23 <sup>c</sup>	3.11 <sup>a</sup>	0.40	0.000
1 - 49	2.26 <sup>b</sup>	2.02 <sup>c</sup>	2.03 <sup>c</sup>	2.74 <sup>a</sup>	0.31	0.000

a,b,c,d: Means with the same superscript on the same line are not significantly different ( $P > 0.05$ ).

P= probability.

RO<sup>-</sup> = control diet; RO<sup>+</sup> = RO<sup>-</sup> + 0.1% Doxycycline<sup>®</sup>; 0.2T = 0.2% of *T. tetraptera*; 0.4T = 0.4% of *T. tetraptera*

The highest BW and BWG were recorded with the antibiotic (RO<sup>+</sup>) irrespective of study phases. Chickens fed on diet supplemented with 0.2% phytobiotic during the growing phase (22-49 days) recorded higher BW and BWG as compared to negative control diet. Chickens fed on diets supplemented with 0.4% *T. tetraptera* recorded the lowest BWG as compared to the rest of experimental diets no matter the study phases.

During the brooding phase (1 to 21 days), 0.2 and 0.4% *T. tetraptera* induced a significant increase in FCR as compared to the negative (RO<sup>-</sup>) and positive (RO<sup>+</sup>) control diets (Table 3). During the growing phase (22 to 49 days) and throughout the production period (1 to 49 days), 0.2% *T. tetraptera* significantly ( $P < 0.05$ ) decrease FCR as compared to 0.4% of *T. tetraptera* and the negative control diet (RO<sup>-</sup>). FCR increased with the increasing level of *T. tetraptera* whatever the phase of the study considered.

**Table 3: Carcass characteristics of broilers fed on diets supplemented with *T. tetraptera* fruit powder**

Carcass parameters (%BW)	Treatments				SEM	P
	RO <sup>-</sup>	RO <sup>+</sup>	0.2T	0.4T		
Carcass yield	74.29 <sup>b</sup>	76.34 <sup>a</sup>	74.32 <sup>b</sup>	69.97 <sup>c</sup>	2.82	0.000
Head	2.28 <sup>a</sup>	2.00 <sup>b</sup>	2.29 <sup>a</sup>	2.41 <sup>a</sup>	0.21	0.000
Leg	3.43 <sup>a</sup>	3.35 <sup>a</sup>	3.71 <sup>a</sup>	3.47 <sup>a</sup>	0.55	0.551
Liver	1.83 <sup>b</sup>	1.61 <sup>c</sup>	1.75 <sup>bc</sup>	1.98 <sup>a</sup>	0.23	0.008
Heart	0.45 <sup>a</sup>	0.46 <sup>a</sup>	0.52 <sup>a</sup>	0.50 <sup>a</sup>	0.48	0.322
Pancreas	0.16 <sup>c</sup>	0.19 <sup>bc</sup>	0.24 <sup>b</sup>	0.29 <sup>a</sup>	0.08	0.002
gizzard	1.57 <sup>bc</sup>	1.42 <sup>c</sup>	1.65 <sup>b</sup>	1.85 <sup>a</sup>	0.23	0.005
Abdominal fat	1.68 <sup>a</sup>	1.38 <sup>a</sup>	1.60 <sup>a</sup>	1.50 <sup>a</sup>	0.67	0.844

a,b: means along the same row with different superscripts are significantly different ( $P < 0.05$ ).

RO<sup>-</sup> = negative control diet; RO<sup>+</sup> = positive control diet; 0.2T = 0.2% of *T. tetraptera*; 0.4T = 0.4% of *T. tetraptera*

The effect of *T. tetraptera* fruit powder on the carcass yield, carcass cuts and offal weight of the broiler are shown in Table 4. Apart from the relative weight of abdominal fat, heart and leg, all other carcass parameters studied ( $P < 0.05$ ) were significantly affected by the antibiotic and *T. tetraptera* powder in the diet. The inclusion of 0.4% *T. tetraptera* fruit powder in the ration induced a significant reduction in carcass yield as compared to all other rations. The highest carcass yield was

recorded with antibiotic followed by 0.2% *T. tetrapleura* fruit powder. The relative weight of pancreas, gizzard and liver increased with increasing level of this phytobiotic in the ration.

**Table 4: Effects of *T. tetrapleura* on production costs of broiler chickens**

Study phases (days)	Treatments				SEM	P
	R0-	R0+	0,2T	0,4T		
Cost of feed intake (FCFA)						
1 - 21	312.02 <sup>b</sup>	395.41 <sup>a</sup>	310.80 <sup>b<sup>c</sup></sup>	304.15 <sup>c</sup>	4.11	0.000
22 - 49	1346.79 <sup>b</sup>	1731.10 <sup>a</sup>	1290.14 <sup>c</sup>	1340.71 <sup>b</sup>	31.32	0.000
1 - 49	1658.81 <sup>b</sup>	2126.51 <sup>a</sup>	1600.93 <sup>c</sup>	1644.85 <sup>bc</sup>	30.60	0.000
Cost of production of kg of live body weight (FCFA)						
1 - 21	429.44 <sup>c</sup>	536.36 <sup>a</sup>	457.11 <sup>b</sup>	522.87 <sup>a</sup>	12.45	0.000
22 - 49	743.17 <sup>c</sup>	803.65 <sup>b</sup>	640.89 <sup>d</sup>	907.15 <sup>a</sup>	27.23	0.000
1 - 49	652.50 <sup>c</sup>	735.23 <sup>b</sup>	594.41 <sup>d</sup>	798.51 <sup>a</sup>	16.22	0.000

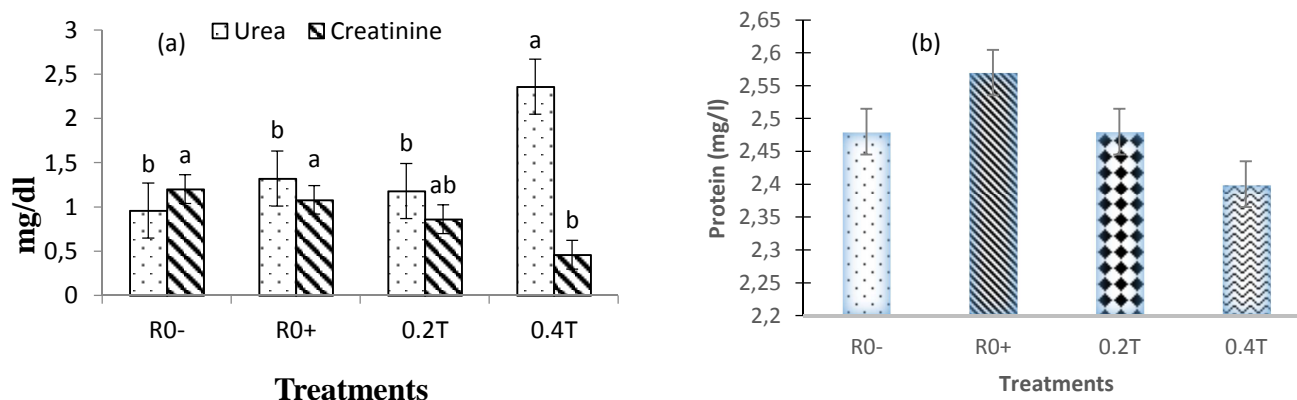
a, b, c: means along the same row with different superscripts are significantly different ( $P < 0.05$ ).

### 3.2 COST OF PRODUCTION

Whatever the phase of the study, the ration supplemented with antibiotic recorded the highest ( $P < 0.05$ ) cost of the feed intake and production of a kg of live body weight. Supplementing the rations with *Tetrapleura tetrapleura* induced a significant ( $P < 0.05$ ) decrease in the cost of feed intake. The same trend was observed with the cost of production of a kg of live body weight with significant ( $P < 0.05$ ) decrease with 0.2% of this phytobiotic as compared to all other treatments in growing phase and all over the study period.

### 3.3 SERUM BIOCHEMICAL PARAMETERS

The supplementation of the ration with *T tetrapleura* fruit powder and the antibiotic induced a significant reduction in the serum content of creatinine as compared to the negative control (Figure 1a). The serum content of creatinine tends to drop while the urea content tends to increase with increasing level of this phytobiotic in the ration. This phytobiotic does not have any significant effect ( $P > 0.05$ ) on serum protein content which tends to decrease with its increasing level in the rations (Figure 1b).



**Figure 1: Variation of (a) urea and creatinine, and (b) protein content of broiler as influenced by incorporation rate of *Tetrapleura tetrapleura* in the diet**

The effect of *T. tetrapleura* fruit powder on serum contents of ALAT and ASAT are shown in Figure 2a and 2b. Ration supplemented with antibiotic induced a significant ( $P < 0.05$ ) increase in of ALAT content as compared to negative control and rations supplemented with phytobiotic. ALAT content tends to decrease with increasing level of this phytobiotic in the ration. The fruit powder of *T. tetrapleura* and antibiotic significantly decreased serum content of ASAT which also tends to decrease with increasing level of this phytobiotic in the ration.

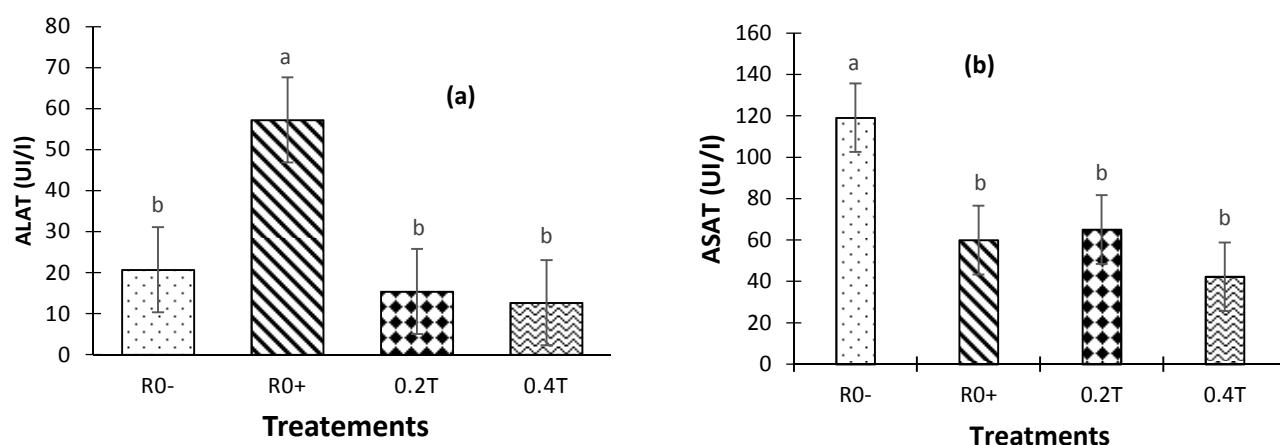


Figure 2: Serum concentration of (a) ALAT and (b) ASAT of broiler as affected by incorporation of *T. tetraptera* in the diet

#### 4 DISCUSSION

This study revealed that 0.2 and 0.4% *T. tetraptera* fruit powder induced significant ( $P < 0.05$ ) decreased in feed intake of about 4.8% and 3.3% respectively as compared to the negative control diet. These results are in close agreement with the findings of Nweze and Nawankwagu [18] which revealed that the inclusion of 4g/l of aqueous extract and 0.04% of fruit powder of this phytobiotic in water and food respectively induced a reduction of feed intake in broiler chickens. The decrease of the feed intake recorded in these studies could be due to its strong odor which might have negatively affected appetibility. The present result contradicted the findings of Ghalib [19] and Galib *et al.* [20] who respectively used anise (*Pimpinella anisum L.*) and pepper (*Capsicum annum*) as feed additives and recorded an increase in feed intake in broiler chickens. These authors explained this feed intake improvement by the appetite stimulative effect of the active ingredients such as the anethol and the borneol contained in anise and the capsaicine in pepper.

During the brooding phase (1-21 days) live body weight (BW) and body weight gain (BWG) tend to decreased with the increasing level of *T. tetraptera* in the ration. This reduction of growth is similar to the results reported by Al Homidan [21] in chicks from 1 to 4 weeks fed on diet supplemented by ginger powder at 60g/kg. Sarica *et al.* [22] also recorded a drop of broilers BW in the first 14 days when fed on diet supplemented by 1g of garlic powder per kg of feed. This may be caused by the antinutritional factor such as tannin, which is responsible for reducing body weight in young birds [23]. Young birds are more sensitive to antinutritional factors than older ones. Throughout the production period and during growing phase, birds fed on ration supplemented with 0.2% of *T. tetraptera* recorded higher BW and BWG compared to 0.4% of this phytobiotic and negative control diet. This improvement of the BW could be due to the antimicrobial and antioxidant activities of this spice. This result is similar to those of Onu [24] and Vivian *et al.* [25] who respectively reported that the powder of ginger and the aqueous extract of ginger significantly improved the growth performances of chickens.

Increasing level of this phytobiotic tends to decrease growth performances of chickens. This result is similar to results obtained by Cross *et al.* [26] and Odoemelam *et al.* [27] who respectively reported that the incorporation of the thyme (*Thymus vulgaris*) and *Ocimum gratissimum L.* essential oil in feed decreased broiler body weight. Odoemelam *et al.* [28] reported that the favorable attributes of spices can be masked when they are used in high proportion because of the marked antinutritional effects generally composed of tannins and saponins. These compounds can affect the use of the nutriment and depress growth [27]. Furthermore, Serrano *et al.* [29] work revealed that even if tannins and saponins inhibit bacteria growth they can also inhibit the enzymes in the digestive tract and reduce the availability of certain minerals and feed proteins for the host.

Diet supplemented with *T. tetraptera* recorded higher feed conversion ratio as compared to other diets during the brooding phase. This can be explained by the fact that feeding young birds with antimicrobial feed additives can disturb the installation of the digestive micro biota leading to a negative effect on the development of the digestive tract [30] and consequently on overall digestion of feed. During the growing phase and throughout the production period, FCR was very low with 0.2% of *T. Tetraptera* fruit powder as compared to negative control diet and ration supplemented with 0.4% of this

phytobiotic. This lower FCR is related to the higher weight gain values recorded in birds fed on 0.2% of this supplement. Windisch *et al.* [6] reported that phytobiotics increased stabilization of intestinal health, animals are less exposed to microbial toxins and other undesired microbial metabolites such as ammonia and biogenic amines. As a result, animals are relatively relieved from immune defense stress during critical situations and there is increase availability of essential nutrients for absorption, thereby helping the animals to grow better within the framework of their genetic potential. Similar results were obtained by Al-Kassie *et al.* [31] with *Capsicum annum* in broiler diet. The increase of broiler FCR on diet supplemented with the highest level (0.4%) of phytobiotic can be related to the large amount of antinutritional content and activity of the secondary metabolites that it contains. Basmacioglu *et al.* [32] reported that feed additives positive effect can be reversed when there are given in high quantities.

The production cost of live body weight was significantly ( $P < 0.05$ ) low with rations supplemented with 0.2% *T. tetraptera*. These results contradict the findings of Nweze and Nawankwagu [17] who reported high production costs with diet supplemented with phytobiotic. The difference can be the consequence of the technological processes of extraction of the phytobiotic done by these authors whereas the phytobiotic used in the present study did not undergo any technological processing which is costly.

No significant difference was recorded between birds fed on 0.2% phytobiotic and negative control diet for carcass yield, head, legs, liver, heart and abdominal fat weight. These results are in closed agreement with those of Dieumou *et al.* [33] and Onu [24] who reported no significant effect on carcass yield and carcass cut when birds were fed on ginger essential oil. The rations supplemented by antibiotic and *T. tetraptera* fruit powder induced an increase in the relative weight of the pancreas as compared to the ration without feed additive. The increase in pancreas weight might suggest that this organ released great quantities of digestive enzymes with increasing level of the phytobiotic in the ration. Gizzard relative weight increased with the increasing level of *T. tetraptera* suggesting an increase in gizzard activity with this phytobiotic in the ration.

There was no significant effect of this phytobiotic on serum contents of transaminases (ALAT and ASAT) and creatinine of chickens fed on 0.2% of phytobiotic and negative control diet. This result suggests that 0.2% of this phytobiotic is not toxic to broiler. In addition, a reduction of the concentration of these indicators of toxicity could be related to the hepatoprotector activity of the *T. tetraptera*. These are in closed agreement with the results of Rehman *et al.* [34] who reported that the mixture of the aqueous extracts of medicinal plants (garlic, aloe vera, ginger) led to a reduction in ALAT and ASAT serum concentration in chickens. Urea concentration of birds fed on 0.2% *T. tetraptera* fruit powder was comparable with that of chickens fed on negative and positive control rations (R0- and R0+). This result is similar to the findings of Vivian *et al.* [25].

## 5 CONCLUSION

Adding 0.2% of *Tetrapleura tetraptera* fruit powder in broiler diet as feed additive improved growth performance stabilized blood components of broiler and reduced cost of production. Hence, *Tetrapleura tetraptera* fruit powder at 0.2% could be used as alternative to the antibiotics growth promoters in poultry production during growing phase.

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