© 2017, Scienceline Publication J. World Poult. Res. 7(1): 27-34, Mar 25, 2017

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Effect of Dietary Mimosa Small Bell (*Dichostachys* glomerata) Fruit Supplement as Alternative to Antibiotic Growth Promoter for Broiler Chicken

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Received: 11 Feb. 2017 Accepted: 18 Mar. 2017

ABSTRACT

There is a growing interest in plant feed additives as a consequence of the antibiotics growth promoters restriction in livestock farming all over the world. This study was designed to evaluate the effect of Dichrostachys glomerata fruit powder on the growth performances of broiler chickens. A group of chickens fed on a basal diet without any supplementation (negative control R0⁻) was compared to three other groups fed on diets supplemented by 0.1% of antibiotic (positive control R0⁺), 0.2% (R0.2) and 0.4% (R0.4) D. glomerata fruit powder respectively. The results revealed a significant decrease in feed intake as compared to the negative and the positive control. The lowest Feed Conversion Ratio (FCR) was recorded with diet supplemented with antibiotic and 0.2% D. glomerata. The Body Weight (BW) and the Body Weight Gain (BWG) of chickens fed on diets supplemented with D. glomerata had an upward trend as compared to negative control diet. Apart from the relative weight of the head which tended to increase in coordination with increasing levels of D. glomerata in feed, this phytobiotic had no significant effect (P>0.05) on carcass characteristics. The increasing level of this phytobiotic tended to decrease serum content of creatinine as compared to the negative and positive control diets. The serum content in ASpartate AminoTransferase (ASAT) tended to increase with the increasing levels of this phytobiotic mean while no significant effect (P>0.05) was recorded on the serum contents of urea, total proteins and ALanine AminoTransferase (ALAT). In conclusion, 0.2% of *D. glomerata* fruit powder can be used to replace antibiotic, for a better growth performances and to produce antibiotics residues free chicken meat.

Key words: Antibiotic, Broiler chicken, Carcass, *Dichrostachys glomerata*, Growth performance, Phytobiotic, Production cost

INTRODUCTION

Due to side effects of the residues in animal products and the resistance developed by bacteria in the poultry farms, antibiotics feed additives have been banned in many countries. As reported by previous studies, up to 81% of the poultry meat and environmental isolates analyzed were resistant to enrofloxacin, ciprofloxacin, tetracycline or erythromycin (Ma De Cesare et al., 2012). As this has negatively affected the poultry profitability, the feed industry has now turned its attention to search for new growth promoter alternatives to antibiotics. Potential alternatives to antibiotics may be found among plant products which have been used for centuries as food and medicines.

Many local plants and spices have also been used as feed additives for poultry all over the world (Alloui et al., 2012; Muneendra et al., 2014). These spices and their extracts represent a new class of growth activators in livestock, but knowledge is still limited concerning their mode of action and their application (Windisch et al., 2008). Some studies have showed that spices contain active substances which have a positive impact on the production performances of domestic animals (Nuhu et al., 2000; Okerulu and Chinwe 2001; Alloui, 2011,

To cite this paper: Kana JR, Mube KH, Ngouana Tadjong R, Tsafong F, Komguep R, Yangoue A and Teguia A (2017). Effect of Dietary Mimosa Small Bell (*Dichostachys glomerata*) Fruit Supplement as Alternative to Antibiotic Growth Promoter for Broiler Chicken. J. World Poult. Res., 7 (1): 27-34.

Odoemelam et al., 2013; Vivian et al., 2015). These compounds act indirectly through their antimicrobial, antioxidant and regulator effects on animal's intestinal microflora (Alloui, 2011). It has also been shown that phytobiotics improve the digestive activity of enzymes and the absorption of the nutrients (Lopez-Boot, 2004; Burt, 2004). The fruit of mimosa small bell (Dichrostachys glomerata) has antioxidant properties (Abdou Bouba et al., 2012), and thorough studies undertaken by Kambizi and Afolayan (2001) emphasizedon an active ingredient called "Apivirine" which is not only an antiviral but also an effective substance in the treatment of gastric ulcers not leaving out the high appetite stimulation effect of this substance. Also, D. glomerata contain flavonoïds and phenols (Abdou Bouba et al., 2012) which are known for their anti-inflammatory and antimicrobial effects (Nuhu et al., 2000; Jane et al., 2014). Okerulu and Chinwe (2001) highlighted the inhibitory effects of these substances on the growth of Staphylococcus epidermidis, Streptococcus viridans and Escherichia coli.

This study was designed to find natural feed additives, available, cheap with no harmful effect on animals, man and the environment in order to mitigate the problems involved in antibiotic feed additives.

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. Where 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).

Birds, dietary treatments and experimental design

A total of 192 day-old Cobb 500 strain broiler chicks were randomly assigned to four experimental diets including negative control 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). The average initial weight of chicks was $39\pm0.04g$. 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*.

Dichrostachys glomerata was bought at the local market, ground in a Harmed mill, sieved and incorporated in experimental diets. Antibiotic (Doxycyclin[®]) used in

positive control diet was bought from a local veterinary pharmacy. Three experimental diets were formulated from a negative control diet (R0-) (Table 1) by incorporating 0.1% of antibiotic (R0⁺), 0.2% (R0.2) and 0.4% (R0.4) of *D. glomerata* fruit powder.

Measurements and blood sampling

Data on feed intake, body weight gain were collected and used to calculate feed to weight gain ratio (FCR). 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 in test tubes without an anticoagulant and left to rest for 12 hours, and the serum was then collected and preserved in the freezer for serum biochemical analysis. Animals were humanly handled in respect of the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Serum biochemical analysis

Serum biochemical analysis (using colorimetric method as prescribed by the Chronolab[®] commercial kits) consisted of the quantification of total proteins, urea, creatinin, aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT). The ASAT and the ALAT were quantified by applying the kinetic colorimetric method of Reitman and Frankel (1972) and Bergmeyer (1972), creatinine was assessed by the colorimetric method described by Newman and Price (1999), serum urea content by the colorimetric method described by Searcy et al. (1967) and Tobacco et al. (1979), total proteins by the Biuret's colorimetric and the bromocresol green methods as described by Gornall et al. (1949).

Production cost

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.

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 the 1964 Declaration of Helsinki and its later amendments.

Statistical analysis

The data were analyzed using Analyses of Variance test by General Linear Model procedure of Statistical Package for Social Science (SPSS 21.0) software. The differences observed were tested using a Duncan's multiple range's test and probability values less than 0.05 were considered as significant (**Steel and Torrie, 1980**).

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	3	6
Fish meal	5	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	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

RESULTS

Performances and carcass traits

The incorporation of *D. glomerata* in the diet significantly improved (P<0.05) growth performances of broiler chickens as compared to the negative control diet (Table 2). During the brooding phase (1 to 21 days), there was no significant (P>0.05) difference between treatment groups for feed intake. Throughout the production period (1 to 49 days), the lowest feed intake was recorded with *D. glomerata* as compared to the negative and positive control diets.

The inclusion of *D. glomerata* in the diet tends to increase BW and BWG as compared to negative control diet (Table 2). The highest BW and BWG were recorded with the antibiotic ($R0^+$) irrespective of the study phases followed by 0.2% of *D. glomerata*. However, BWG of chickens fed on diet supplemented with 0.2% *D. glomerata* during the brooding phase (1-21 days) were

comparables to the control diets. During the finisher phase (22 to 49 days) and throughout the experimental period (1 to 49 days), BWG had an upward trend with inclusion of *D. glomerata* in the diet.

During finisher phase and throughout the production period, FCR was significantly higher with the negative control diet without any supplement as compared to diets supplemented with antibiotic and *D. glomerata*. However, the lowest FCR were recorded with antibiotic and 0.2% *D. glomerata*.

The effects of *D. glomerata* incorporation level on carcass characteristics of broiler chickens are presented in Table 3. Except for the relative weight of the head, all the carcass parameters were not significantly (P>0.05) affected by the inclusion of this phytobiotic in the diet.

Cost of production

Irrespective of the study phase, dietary inclusion of D. glomerata led to a significant decrease (P<0.05) in the

cost of production as compared to the positive control diet (R0+) which recorded the highest cost of feed intake and cost of production of a kg of chicken (Table 4).

Serum biochemical parameters

The effects of *D. glomerata* inclusion level on serum biochemical parameters of broiler chickens are presented in Table 5. Serum content in protein, urea and ALAT were not significantly (P>0.05) affected by the inclusion of *D*.

glomerata in the rations whereas creatinine content significantly decreased (P<0.05) with increasing level of this phytobiotic. The ration R0.4 containing the highest level of *D. glomerata* induced the lowest level of creatinine as compared to the positive and the negative control diets. The reverse trend was recorded in serum content of ASAT with the highest level of this phytobiotic as compared to the control diets (R0⁻ and R0⁺).

Table 2. Growth performances of broiler chickens as affected by Dichrostachys glomerata from one to 49 days

Study phases		Rations	SEM	n			
(days)		R0 ⁻	R0 ⁺	R0.2	R0.4	SEM	Р
Feed intake	1 – 21	1003.29 ^a	1024.38 ^a	988.42 ^a	994.06 ^a	6.36	0.204
(g)	22 - 49	4746.38 ^{bc}	4825.37°	4602.53 ^{ab}	4509.66 ^a	39.85	0.006
-	1-49	5749.67 ^{bc}	5849.74 ^c	5590.95 ^{ab}	5503.73 ^a	42.22	0.003
Body weight							
(g)	1 - 21	763.92 ^{ab}	774.60 ^b	742.29 ^{ab}	732.38 ^a	6.59	0.070
(6)	22 - 49	2584.00 ^a	2929.83 ^b	2702 ^a	2604.97 ^a	41.42	0.001
Body weight	1 – 21	726.96 ^{ab}	737.64 ^b	705.33 ^{ab}	695.42 ^a	6.59	0.070
gain (g)	22 - 49	1820.08 ^a	2155.22 ^b	1959.71 ^a	1872.59 ^a	39.73	0.002
	1 – 49	2547.04 ^a	2892.87 ^b	2665.04 ^a	2568.01 ^a	41.42	0.001
Feed	1 01	1 208	1.201	1 408	1 428	0.01	0.001
conversion	1 - 21	1.38 ^a	1.39 ^a	1.40 ^a	1.43 ^a	0.01	0.281
ratio	22 - 49	2.62 ^b	2.24^{a}	2.35^{a}	2.41 ^a	0.04	0.04
	1 - 49	2.26 ^c	2.02^{a}	2.10^{ab}	2.14 ^b	0.03	0.002

a, b, c: Means with the same superscript on the same row are not significantly different (P>0.05). SEM= standard error of mean. p= probability, $\mathbf{R0}^{+} = \text{control diet}$ (without any additive); $\mathbf{R0}^{+} = \mathbf{R0}^{-} + 0.1\%$ of Doxycycline[®]; $\mathbf{R0.2} = \mathbf{R0}^{-} + 0.2\%$ of fruit powder of *D. glomerata*; $\mathbf{R0.4} = \mathbf{R0}^{-} + 0.4\%$ of fruit of powder of *D. glomerata*

Table 3. Carcass characteristics of broilers fed on diets supplemented with the fruit powder of D. glomerata at 49 days

Parameters (%BW)		Tı		P		
	R0 ⁻	$\mathbf{R0}^+$	R0.2	R0.4	SEM	Р
Carcass yield	74.29 ^a	76.66 ^a	74.61 ^a	74.60 ^a	43.92	0.218
Head	2.27 ^b	2.01 ^a	2.17 ^{ab}	2.24 ^b	1.40	0.044
Leg	3.43 ^a	3.36 ^a	3.36 ^a	3.59 ^a	3.10	0.705
Liver	1.79 ^a	1.62 ^a	1.54 ^a	1.77 ^a	1.04	0.178
Heart	0.47^{a}	0.46^{a}	0.44 ^a	0.45 ^a	0.48	0.968
Pancreas	0.16^{a}	0.19 ^a	0.14 ^a	0.20^{a}	0.30	0.292
Gizzard	1.57 ^a	1.43 ^a	1.50 ^a	1.62 ^a	0.99	0.945
Abdominal fat	1.68 ^a	1.39 ^a	1.48 ^a	1.27 ^a	2.61	0.195

a, b: means on the same row with different superscripts are significantly different (P < 0.05). SEM= standard error of mean. P= probability. R0⁻ = control diet (without any additive); R0⁺ = R0⁻ + 0.1% of Doxycycline[®]; R0.2 = R0⁻ + 0.2% of fruit powder of *D. glomerata*; R0.4= R0⁻ + 0.4% of fruit of powder of *D. glomerata*

Table 4. Effects of Dichrostachys glomerata on production costs of broiler chickens from one to 49 days

Study phases		Treatments					D
(days)		R0 ⁻ R0 ⁺ R		R0.2	R0.2 R0.4		Р
Cost of feed intake	1 - 21	312.02 ^a	395.41 ^b	309.37 ^a	313.13 ^a	9.54	0.00
(FCFA)	22 - 49	1346.79 ^a	1731.10 ^b	1315.17 ^a	1297.66 ^a	46.81	0.00
	1 - 49	1658.81 ^a	2126.51 ^b	1624.55 ^a	1610.79 ^a	56.05	0.00
Cost of production of kg	1 - 21	429.44 ^ª	536.36 ^c	438.57 ^{ab}	450.55 ^b	11.32	0.00
of live weight (FCFA)	22 - 49	743.17 ^b	803.65 ^c	671.38 ^a	693.61 ^{ab}	15.05	0.00
	1 - 49	652.49 ^b	735.23 ^c	609.66 ^a	627.67 ^{ab}	13.18	0.00

a, b. c: means along the same row with different superscripts are significantly different (P < 0.05). SEM= standard error of mean. P= probability. R0⁻ = control diet (without any additive); R0⁺ = R0⁻ + 0.1% of Doxycycline[®]; R0.2 = R0⁻ + 0.2% of fruit powder of *D. glomerata*; R0.4= R0⁻ + 0.4% of fruit of powder of *D. glomerata*

Table 5. Serum biochemical parameters of broilers fed on diets supplemented with Dichrostachys glomerata

Parameters	Treatments		SEM	Р		
	R0 ⁻	R0 ⁺	R0.2	R0.4		
Protein (g/dl)	2.48^{a}	2.57 ^a	2.45 ^a	2.61 ^a	0.27	0.604
Urea (mg/dl)	1.02 ^a	1.32 ^a	0.78^{a}	1.31 ^a	0.37	0.146
Creatinin (UI/L)	1.20 ^b	1.08b	0.81 ^{ab}	0.56 ^a	0.33	0.020
ALAT (UI/L)	19.83 ^a	15.83 ^a	13.28 ^a	21.33 ^a	5.60	0.229
ASAT (IU/L)	123.67 ^a	121.36 ^a	204.00 ^b	220.00 ^b	51.52	0.002

a, b, c: means on the same row with different superscripts are significantly different (P < 0.05). SEM= standard error of mean. P= probability. $R0^{\circ}$ = control diet (without any additive); $R0^{\circ}$ = $R0^{\circ}$ + 0.1% of Doxycycline[®]; $R0.2 = R0^{\circ} + 0.2\%$ of fruit powder of *D. glomerata*; $R0.4 = R0^{\circ} + 0.4\%$ of fruit of powder of *D. glomerata*

DISCUSSION

Positive features of plant extract, essential oils and spices are being increasingly used as feed additives in poultry farms. They contain active substances and their activity vary to a great extent between plant species, depending also on the harvesting period, technology of drying and extraction processes. Beneficial effects of plant extract and spices related to their bioactive compounds such as flavonoids that affect feed intake in poultry were reported in many recent studies (Khaligh et al., 2011; Khan et al., 2012). In the present study the Dichrostachys glomerata supplementation did not improve broiler feed intake. It induced a significant (P<0.05) decrease as compared to the control (positive and negative) diets. This result contradicted the findings of Herawati (2010), who reported that the incorporation of 2% of ginger (Z. officinal) in broiler feed increased their feed intake. The decrease in feed intake in the present study could be due to the strong odor of this spice. As reported by Hernandez et al. (2004), optimization of feed intake with feed additives from plant origin is controversial and depends on the amount and duration of administration.

Throughout the study period, the BW and the BWG of the birds fed on diets supplemented with the fruit powder of *D. glomerata* were higher as compared to chickens fed on the negative control diet but lower than chickens fed on positive control diet supplemented with antibiotic ($R0^+$). This result is similar to the findings of Vivian et al. (2015) who reported that the aqueous extract of ginger markedly improved the growth performances of chickens. The present result contradicted the findings of El-Deek (2012) who reported that the incorporation of Hot Pepper (*Capsicum annuum*) at 1.5g/kg in broiler feed induced an increase in BW and BWG of about 21.2%

supplemented with the antibiotic (Oxytetracycline). The improvement in BWG with this spice could be attributed to their antimicrobial properties and impact on gut function (Alloui, 2011; Jane et al., 2014). In fact, this spice contains phenolic and flavonoïds compounds which act by forming the complexes with many proteins, cause the destructuration of the bacterial membranes, making unavailable certain substrates for the bacteria and inactivate bacterial enzymes (Abdou Bouba et al. 2012). Thus the reduction of the microbiota could lead to a greater availability of some nutriments for the host and consequently improve BWG. This is in agreement with Mcmullin (2000) who observed that the growth promoting effect of most herbs and extracts of spices act by killing parasites that hinder digestibility and growth performance of birds. Moreover, the secondary metabolites present in the spices exhibited antioxidant properties and it could be probably the case with this spice. Several studies reported that phytobiotics improved intestinal health, animals are less exposed to microbial toxins and other undesired microbial metabolites (Nuhu et al. 2000; Kambizi and Afolayan, 2001). As a result, animals are relatively relieved from immune defense stress during critical situations and there is increased availability of essential nutrients for absorption, thereby helping the animals to grow better within the framework of their genetic potential. The low performances recorded with the fruit of D. glomerata compared to antibiotic could be explained by the presence of the high content of tannins in this spice (281mg/100g) (Abdou Bouba et al., 2010) which would have unably used digestive nutrient like protein by chickens. Odoemelam et al. (2013) reported that the favorable attributes of spices can be masked by tannins which affect the use of the nutrients and depress growth.

Incorporation of phytobiotic in the diet have led to a significant (P<0.05) reduction of FCR as compared to negative control diet (R0-). This result is in close agreement with the findings of Al-harthi (2002) who recorded a decrease in FCR with the inclusion of 0.3% of *Capsicum annum* in broiler feed. The decrease in feed conversion ratio can be understood because of the increase in the body weight gain of birds fed on *Dichrostachys glomerata*. Furthermore, this is in agreement with Mcmullin (2000), Nuhu et al. (2000), Okerulu and Chinwe (2001), Kambizi and Afolayan (2001) and Abdou Bouba (2012) who reported that most herbs and extracts of spices work as growth promoters by killing parasites that hinder digestibility and growth performances of birds.

The serum content of urea and proteins were not affected (P>0.05) by the supplementation of feed with D. *glomerata*. This suggests that the inclusion of D.

glomerata fruit powder in broiler diet does not have harmful effects on kidney function (serum rate of urea) and the immune system (serum protein rate). This result contradicts the study of Zhang et al. (2009) which revealed that the incorporation of the powder of ginger (Z. officinal) in broiler feed increased their total protein ratio. It can be explained by the presence of active substances such as gingerole, shogaoles, gingerdiole and the gingerdione in ginger (Kikuzaki and Nakatani, 1996; Zhang et al, 2009; Zhao et al., 2011) which are absent in D. glomerata fruit. In addition, the serum creatinin content rather decreased with D. glomerata in the feed. This fall materializes the presence of active substances in this phytobiotic, allowing the correct function of the kidneys. The serum contents of ALAT (alanine amino-transferase) and ASAT (aspartate amino-transferase), were not significantly affected by the incorporation of 0.2 and 0.4% of D. glomerata in their diet. However, the serum content in ALAT and ASAT had an upward trend with the increasing rate of this additive in feed. This observation contradicted the finding of Rehman et al. (2011) who reported that feeding broiler with a mixture of aqueous extracts of medicinal plants induced a reduction in ALAT and ASAT ratios. This contradiction can be due to the multitude of the active compounds in the mixture of the extracts used by these authors which could have affected liver function.

CONCLUSION

This study revealed that 0.2% *D. glomerata* fruit powder is a profitable feed supplement since it is produced at a relative low cost meat and can then be used as good alternative to antibiotics growth promoters in broiler diet. The dietary supplementation of *D. glomerata* powder can lead to the production of antibiotics residues free chicken meat as demanded by consumers.

Competing interests

The authors declare that they have no competing interests.

Author's contributions

Ngouana, Komguep, Yangoue and Tsafong went to the field to carry out the research and collect the samples. Kana supervised the overall research work. Mube wrote the first draft before being revised by Kana 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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