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Therapeutic activity of *Thonningia sanguinea* aqueous extract, Vahl on an experimental colibacillosis in chicken

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ABSTRACT

Broiler breeding is faced with serious diseases, including colibacillosis, which is the leading cause of antibiotic therapy and the second leading cause of mortality in poultry after salmonellosis. It is caused by avian pathogens of *E. coli*. The objective of this study is to evaluate the effect of the aqueous extract of *Thonningia sanguinea* on certain therapeutic parameters in broiler chickens during experimental colibacillosis compared to a usual antimicrobial against colibacillary diarrhea (colistin). For each parameter, three lots of chicken meat were used: two treated batches, one with the plant extract and the other with colistin and one untreated contaminated batch (control). The study was spread over two weeks and for one week each chicken in the treated batch absorbed 500 mg of plant extract daily. The results show that the aqueous extract of this plant causes an improved clinical index, increased mean weight gain, reduced organ damage, elimination of germs in affected organs, and decreased mortality compared to the control group. On all the parameters, other than the moisture content, the plant extract had a more beneficial effect than colistin. Under these conditions, this plant could be used as a veterinary product to control avian colibacillosis.

Keywords: *Thonningia sanguinea*, *E. coli*, Colibacillosis, Broiler chickens.

INTRODUCTION

Meat production in the world is increasing increasingly to meet the high demand for protein. This is linked to the rapidly growing demographics [1]. Poultry has a dominant position in the meat sector with world production of 112.1 million tonnes in 2015 according to the [2]. It will account for about half of meat production in the next 10 years. This expansion will be mainly driven by developing countries, which will contribute about 73% to additional production [3]. Chicken is de facto the cheapest meat to produce and cheapest meat on the market [4]. In fact, the short production cycle of these animals allows breeders to adapt quickly to market signals. This involves rapid improvements in genetics, animal health and dietary practices [5]. In Côte d'Ivoire, chicken production almost doubled in just four years, from 22,364 tonnes in 2011 to 44,451 tonnes in 2015 [6].

However, the rise of the poultry sector throughout the world is confronted with various serious pathologies. Among these diseases is colibacillosis caused by pathogenic strains of *Escherichia coli*. It is a very common bacterial infection in poultry farming, which decimates avian farms and causes considerable economic losses to livestock farmers [7]. Chickens suffering from this disease are usually treated with antibiotics [8].

On the other hand this chemotherapy presents many deficiencies. Indeed, in recent years, avian colibacilli have become increasingly resistant to these drugs [9]. The regular appearance of this resistance is linked to the anarchic use of antibiotics and the virulence factors present in pathogenic strains [10].

Faced with these therapeutic failures, the use of phytomedicines offers itself as a solution to be explored. It was in this context that we evaluated the therapeutic effects of aqueous extracts of *Thonningia sanguinea*, a plant of African tropical forests, on experimental colibacillosis in broiler chickens.

MATERIALS AND METHODS

Plant Material

It consists of the inflorescences of *Thonningia sanguinea* harvested at Sandegue in the eastern region of Côte d'Ivoire (West Africa) and authenticated by the National Floristic Center (NFC) of the Felix

Houphouët-Boigny University in Cocody-Abidjan.

Microbial material

A multi-resistant avian pathogenic strain of *Escherichia coli*, isolated from liver of chickens with colibacillosis which was supplied by the central veterinary laboratory of Bingerville. This strain was tested on a day-old chick to confirm its pathogenicity [11].

Animal equipment

We used 60 chicks of one (1) day of raceCobbs divided into three batches of 20 chicks each.

Lifting equipment

It consists of three large compartments each receiving a batch of 20 chickens. Each compartment made of wood and wire mesh contains drinking troughs and feeders.

Preparation of the plant extract

The inflorescences of *T. sanguinea* were washed, cut and dried out of the sun for two weeks. Once dried, these plant elements are ground. Thus, 20 g of this powder are dissolved in 2 L of distilled water. The mixture is homogenized at room temperature using a magnetic stirrer. The homogenate is filtered seven times on hydrophilic cotton and once on 3 mm Wahtman paper. The filtrate obtained is evaporated under vacuum at 30 °C in a Buchi-type rotavapor. The evaporate is lyophilized and made dry and then recovered in the form of a powder which constitutes the total aqueous extract [12] and [13]. The vegetable extract thus obtained is stored in the refrigerator to serve as drinking water for the chicks to be treated.

Method of breeding

Sixty (60) chicks of flesh were divided into three (3) batches of twenty (20) chicks:

- Lot 1: contaminated and untreated.
- Lot 2: contaminated and treated with aqueous extract of *T. sanguinea* at 10 mg/mL.
- Lot 3: contaminated and treated with a standard avian anti-diarrhea, colistin

All chickens were fed identically with the traditional broiler chicken feed provided by the Cattle Feed Manufacturing company in the Ivory Coast (FACI).

Induction of colibacillosis in chicks

After incubation for 18-24 hours, 3 colonies of *Escherichia coli* are used to inoculate 20 mL of Muller-Hinton broth which is then incubated at 37° C for about 4 hours so as to obtain an estimated bacterial load of 10^7 bacteria/mL. Then, 0.1 mL of this suspension is taken to subcutaneously inoculate each chick of the two batches at the neck after one week ofacclimatization on the farm [14].

Treatment of chicks

The animals of lot 2 are treated with the plant extract as soon as the first signs of colibacillosis appear. The treatment lasts one week during which each of the chicks receives daily 50 mL of the *T. sanguinea* extract at 10 mg/mL, or about 500 mg in the form of drinking water after thirsting from 18h to 7h in the morning [15].

The animals of lot 3 receive the usual standard antibiotic colistin in the form of sulfate. This antibiotic was administered for 5 days via drinking water at the recommanded dose of 37.5 mL of solution per tonne of live weight ie 75000UI /kg of weight / day of traitement [16].

Medicated water is supplied to chickens in siphotic drinking troughs of sufficient capacity to cover the watering needs of the animals for 24 hours. It is renewed every day.

Description of the clinical indices of the animals and moisture content of the droppings

A clinical examination is carried out in each batch of animals when they arrive on the farm to ensure that they do not show any signs of colibacillosis. During the experiment, the behavior of the animals is described daily and the moisture content of the droppings is also determined in each batch according to the method of [15] from the following formula:

$$\textbf{Moisture content} = (Ph - Ps) \times 100/Ph$$

With **Ph:** wet weight and **Ps:** dry weight after drying for 24 hours at 70°C.

At the end of the experiment, the mortality rate is also determined in each batch.

Determination of the average weight gain of chickens.

The chickens were weighed individually before the experiment on D_0 (when they arrive) and every 3 days during the experiment. The average weights of chickens per lot are determined from the following formula:

$$P_m = 1/N \times \Sigma P_i$$

With P_m: Average weight of chickens (g)/lot; ΣP_i:Sum of individual weights of chickens per batch (g); **N:** Number of chickens per lot

These average batch weights calculated in this way were used to determine the mean weight gains of chickens per batch as follows:

$$GP_m = P_{mj} - P_{mp}$$

WithGP_m: Average weight gain (g)/lot; P_{mj}: Average weight of the day (g); P_{mp}: Previous average weight (g)

Search for lesions

An autopsy was carried out on 3 chickens per batch on the following days D_0, D_1, D_4, D_7 and D_{14} according to the standard procedure used at the central veterinary laboratory in Bingerville (Côte d'Ivoire) in order to detect possible changes in the lesion.

Search for pathogenic colibacilli in chickens

At the same dates of autopsies, liver samples from autopsied animals are taken. Indeed 1 g of liver is ground in 10 mL of buffered peptone water allowing a better development of the microflora. There is then a 10^{-1} mother suspension. Then, from this broth, double dilutions were made with sterile distilled water to obtain concentrations 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} using disposable pipettes. For each dilution obtained, 3 Petri dishes containing Mac Conkey agar, a selective medium for enterobacteriaceae are inoculated. Seeding of the Petri dishes is done by spreading 0.01 mL of each dilution using a sterile spreader. The plates are then incubated in an oven at 37°C for 18 to 24 hours. After incubation of the dishes, 2 colonies were taken to make a suspension with sterile distilled water. From this suspension and using a sterile Pasteur pipette, the API 20 E galleries specific to enterobacteriaceae allowing the search for colibacilli are seeded.

Statistical analysis of results

The Values of moisturecontent of the droppingsand weight gain were accompanied by the standard error on the mean (mean ± ESM).

RESULTS

Clinical indices of animals in different batches

On arrival in the farm and after a week of acclimatization, the day-old chicks all presented a normal clinical state and a great vivacity. Table I shows the behavior of the animals of the three batches, 48 hours (D1) after the contamination and during the treatment. The signs of colibacillosis which appear persist until the end of the experiment in the control batch 1, whereas from the second day of treatment there is an improvement in the condition of the animals of the treated batch 2, full vivacity and increased food consumption at the end of the treatment. Compared to lot 2, it is necessary to wait until the fourth day to note an improvement in the state of the chickens of lot 3 which continues until D7 with a total vivacity.

Table 1: Behavior of the animals of the different batches.

Lots	**Days of experimentation**							
	D_0	**D_1**	**D_2**	**D_3**	**D_4**	**D_5**	**D_6**	**D_7**
Lot 1	Normal, lively	Inappetence, ruffled feathers, drooping of the head, somnolence.	Little alive, somnolence inappétence,	Idem	Idem	Idem	Idem	Idem
Lot 2	Normal, lively	Inappetence,lower consumption, ruffled feathers,	Little alive	lively	Lively	lively	Increase in consumption, lively	Increase in consumption, lively
Lot 3	Normal, lively	Ruffled feathers, inappetence, head loss, stress and isolation	Idem	Idem	Stress, feathers slightly shaved	Idem	Little alive	lively

The moisture content of or the droppings

Figure 1 shows that the moisture content of lot 1 increases considerably from 24.12±0.22% to 88.45±0.77% from D_0 to D_7. In comparison to lot 1, lot 2 recorded high moisture levels on the first two days of treatment (60,11±0.59% and 64.07 ±0.33%) and on the third day (59±0.55%). After the third day, this moisture content drops considerably up to 19±0.5% on D_7. Compared to lot 2, the same trend was recorded in lot 3 with stability in the last two days at 21.3 ±0.22%. There is no significant difference between the moisture levels of the treated batches. The decrease in the moisture content in the two treated lots is also done with the appearance of solid droppings compared to the lot 1where the diarrhea persists until the end of the experiment.

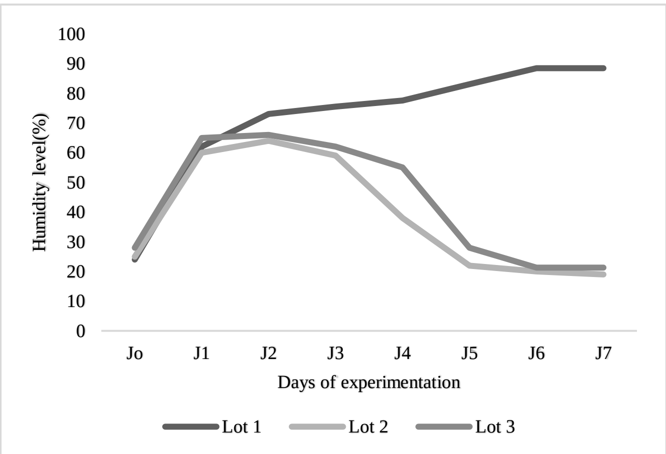

Figure 1: Moisture curves in the three lots of chickens

Weight gain

Figure 2 shows the variation in mean weight gain in batches just before the disease, during and after treatment. These results show a decrease in the average weight gain of the chicks of lot 1, lot 2 and lot 3 from the onset of the first signs of 14 ± 0.41 g to 8 ± 0.83 g and 17.88 ± g to 11.56 ± g from 19 ± g to 11 ± g up to D7. From this date the average weight gain of lot 1 remains constant whereas that of the last two batches treated with *T. sanguinea* and colistin increases considerably up to 70 ±0,33g for lot 2 and 59±0,28 g for lot 3 to the end of the experiment on D16. There was a significant difference between the mean weight gains of the treated lot with *T. sanguinea* and the lot treated with colistin. There is also a significant difference between the treated batches and the untreated batches.

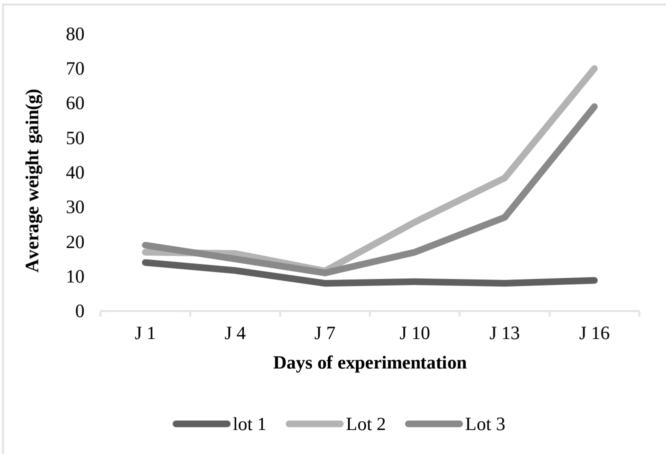

Figure 2: Average weight gain curves of the two lots of chickens

Search for lesions

Table 2 summarizes the results of autopsies performed in the three lots on dates D_0, D_1, D_4, D_7, D_{14}. The lesions persist in the animals of lot 1 from the beginning to the end of the experiment, whereas in batch 2 there is an attenuation of the lesions which disappear at the end of the treatment. In the latter batch, no lesions were observed on Day 14, one week after discontinuation of treatment.

On the other hand, in lot 3, there is a persistence of the lesions with mainly pericarditis and nephritis showing the ineffectiveness of colistin on these lesions.

Table 2: Lesions and their evolution in the two batches

Lots	Days of experimentation				
	D_0	D_1	D_4	D_7	D_{14}
Lot 1	No lesion	Subcutaneous tissue congested, perihepatitis, fibrinous, nephritis, pneumonia	Nephritis, hypertrophied purse of Fabricius, fibrinous pericarditis, pneumonia.	Fibrinous perihepatitis, peritonitis, fibrinous pericarditis, caeca hypertophied, mild pneumonia, haemorrhagic enteritis	Idem
Lot 2	No lesion	Wet subcutaneous tissue, fibrineous pericarditis, haemorrhagic hepatitis, néphromegaly	Mild nephritis	No lesion	No lesion
Lot 3	No lesion	Congestion of cadavers, congestion of subcutaneous connective tissue, nephromaemia, pneumonia	Fibrinous pericarditis, congestive hepatitis, nephritis, hypertrophied caeca	Nephritis, pericarditis	Nephritis, pericarditis

Search for germs in animal organs

Table 3 shows the presence or absence of *E.coli* in the seeded samples. In lot 1, a diseased and untreated control and in the third lot diseased and treated with colistin, *E.coli* was present in the liver of the animals from D_0 until the end of the experiment showing thus the inefficiency of colistin on intracellular germs. Whereas at D_7 (the end of treatment) no colony was observed on the boxes of lot 2 treated with the plant extract.

Table 3: Presence or absence of *E. coli* in the lesions of the animals of the three lots

Day \ Lot	Lot 1	Lot 2	Lot 5
D_0	–	–	–
D_1	+	+	+
D_4	+	+	+
D_7	+	–	+
D_{14}	+	–	+

+: Presence of *E.coli* in the lesions; -: Absence of *E. coli* in lesions

Death rate

The mortality rates were 15%, 5% and 10% respectively for lot 1, lot 2 and lot 3. The mortality occurred exclusively on day 1, that is to say one day after the contamination for lot 2 treated with the plant extract whereas in the untreated lot 1 and lot 3 traited with colistin, mortality occurred on day 1 and continued throughout the experiment.

DISCUSSION

In this study, our objective was to treat avian experimental colibacillosis with the total aqueous extract of T. sanguinea and to compare the effects of this plant with those of colistin, a reference colibacillary anti-diarrhea.

Animals contaminated and treated with this plant extract (lot 2) experienced an improvement in clinical index, an increase in mean weight gain, reduction of lesions, elimination of colibacilli in the liver and a decrease of mortality.

Treatment of chicks with *T.sanguinea* extract reduced the moisture content of chick droppings in lot 2 from 60.11±0.59% on the first day of treatment (D1) to 19±0.5% on D7. Statistical analysis showed that there was no significant difference between these results and those observed with lot 3 treated with colistin. On the other hand, there is a significant difference between the results of the treated batches and those of the control. These results confirm the effect of this plant against infectious diarrhea [15] and [17]. Indeed, these authors treated with the aqueous extracts of *T. sanguinea* experimental diarrhea respectively due to *Salmonella enteritidis lysotype 6* and parasites of the genus *Emeria*.

Furthermore, in animals treated with the plant extract, the reduction in diarrhea is accompanied by an increase in mean weight gain and a return to liveliness, contrary to that observed in the animals of the control group and of the lot treated with colistin. These positive results with *T. sanguinea* are similar to those found by [18] and [19] who reported the beneficial effects of extracts of this plant on weight growth of healthy hens and salmonellosis hens, respectively. In the same vein, [17] in an experimental study obtained an increase in the weight of chickens with coccidiosis with this plant.

This plant extract also reduced in batch 2 the lesions caused by colibacillosis with total disappearance of the responsible germs in the liver, unlike the two other batches where there was persistence of these germs in this organ. *T. sangui*nea would therefore have an anti-*E. coli* action in vivo in aerial organs in broiler chickens. This would also justify the improvement of the clinical index which is related to lesions of organs according to [20].

These results could be explained by the antioxidant properties of certain molecules contained in the extracts of *T. sanguinea*. Indeed [21] have demonstrated these properties by isolating two antioxidant molecules from these extracts, gallic acid and brevifolin. In the same vein, two other antioxidant molecules named *Thonningianin A and B* were extracted from this plant by [22]. The interest of antioxidant substances in reducing tissue damage has already been reported by [23] and [24]. These authors have demonstrated the role of antioxidants brought by the diet against infections and in improving the functioning and lifetime of immune cells. These antioxidant molecules of *T. sanguinea* could also act to inhibit the expression of virulence genes. According to [25], these genes are responsible for the establishment and development of the disease in chickens.

Moreover, triphyochemical studies of this plant carried out by [26] have also demonstrated the presence of tannins and saponins, two other chemical groups that improve the zootechnical performance of chickens according to [27].

Other plant extracts have already been used by researchers as a palliative solution to antibiotics in the control of avian colibacillosis. In this context, [28] by biochemical methods and scanning electron microscopy showed that essential oils of clove and oregano act through their major phenolic constituents to cause alterations of *E. coli* membranes. These oils have an antimicrobial, anti-inflammatory and antioxidant effect. This allows them to stimulate the animal's defenses and reduce damage caused by free radicals [29]. In addition, the animals in lot 2 who were treated with the plant extract, even one week after discontinuation of the treatment, did not show clinical signs of recurrence or new tissue lesions. This shows a complete healing of the treated animals and gives a lasting appearance to the effect of this plant on these animals.

Moreover, the mortality rate has also been improved by this plant. Indeed, the 5% mortality observed in the treated animals occurred during the 24 hours after the contamination and before the treatment, compared with 10% in the colistin-treated batch and 15% in the control that occurred throughout the experiment. This is in agreement with the results of [15] which obtained with this plant an elimination of mortality and morbidity in the laying hen during experimental salmonellosis with similar rates.

CONCLUSION

At the end of this study, the results show that the total aqueous extract of *T. sanguinea* allows an improvement of the clinical index, an increase in weight gain and a halting of the lesions caused by *E.coli* with total elimination of this germ in the liver in broiler chicken. This extract also reduces the mortality and morbidity of chickens with colibacillosis. Compared to colistin which is a reference antimicrobial against avian diarrhea, *T. sanguinea* has shown a more beneficial effect especially on average weight gain, lesions and elimination of germs in the liver.

In the remainder of our work, we intend to carry out a molecular characterization of the colibacils isolated from the liver of the animals contaminated by PCR in order to make sure that they are the same ones that caused the disease.

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