**Moringa Oleifera Leave:**
Hydro-Alcoholic Extract and Effects on Growth Performance of Broilers

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**Abstract:** After the ban in 2006 of the use of antibiotic growth promoter, the search for an alternative led to the utilization of plants like *Moringa oleifera* Lam. Leaves of this plant are known to have an important component of macronutrients (protein, energy, amino acids), of micronutrients (vitamins, minerals…) and of anti-nutritive factors such as polysaccharides, tannins, saponins, phytates etc. In the aim to give more knowledge about it, leaves are collected from Akouramé (Vo district in Togo), dried, pulverized and soaked in ethanol-water (50/50). The mixing obtained is homogenized, filtered and evaporated to obtain hydro alcoholic extract. This extract was used to determine its contents in some chemical groups such as total phenols (4.2%), tannin (2.36%), total flavonoids (0.2%) and polysaccharides (21.1%). In addition, a total of 615 day-old broilers (Ross) were divided at random into 3 groups (M0, M1 and M2) fed, respectively with diet 1 (0%), diet 2 (1%) and diet 3 (2%). During the assay, witch lasted for 4 weeks, 15 chicks of each group were slaughtered weekly to collect and weight liver, pancreas, spleen, bursa and thymus. At the same moment, body weight, feed intake, body weight gain and feed conversion ratio were determined. At 28th day, chicks of groups M1 and M2 grew better and have better feed conversion than chicks of groups M0. The same trend is followed by relative organ weights. It can be concluded that *Moringa oleifera* leaves incorporated at 1 and 2% in feed can improve growth and the lack of significant difference between 1 and 2% could be attributed to the high content of diet 3 in anti-nutrients especially saponins that impair the digestion and absorption of nutrients especially lipids.

**Key words:** *Moringa oleifera* leaves, broiler chicks, chemical groups, body weight, feed conversion, relative organ weights

**INTRODUCTION**

Over several years, antibiotics are widely used in chicken for therapeutic use to treat diseases, prophylactic use to prevent infections or as growth promoters to improve feed efficiency and performance. But, resistance to antibiotics associated with the use of antibiotics in animals leads to the risk of transfer of antibiotic-resistant genes to human pathogens (Gould, 2008). Also, there is the issue of reduced efficacy of antibiotic therapy in chicken infected with resistant bacteria. The large use of oral medication in chicken may increase the incidence of unacceptable residues in eggs or meat. Such residues may be reduced by establishment and adhering to withdrawal periods for eggs or before slaughtering. According to the World Health Organization (WHO) (2008), the use of antimicrobials in food animals is a public health issue. As alternatives to the use of antibiotics as growth promoters, probiotics were developed and incorporated in poultry feed as a potential tool for reducing intestinal contamination with disease-causing and food-borne bacteria. Recently, a lot of interests were focussed on investigation for alternatives to antibiotic growth promoters. Various plants extracts, especially essential oils, have been studied for their antimicrobial abilities (Giggs and Jacob, 2005). A review of available literature shows that *Leucena leucocephala*, *Glinicidia sepium*, *Sesbania sesban* and *Manihot esculenta* have been widely used in feeding non-ruminants and especially poultry resulting in improvement of their productivity (D’Mello et al., 1987). However, plants may contain some nutrients or anti-nutritive factors that might affect positively or negatively production parameters. Thus, any plant that can be used for its abilities to improve productivity should be investigated in order to determine the limits of its incorporation in animal feed. In tropical regions, *Moringa oleifera* leaves are widely used traditionally for its antimicrobial abilities (Suarez et al., 2005) and its pharmacological properties (Mehta et al., 2003). This plant is known to contain 23% of crude
protein, 12 MJ/Kg of metabolizable energy and to possess 79.7% of digestibility (Becker, 1995). It also contains sufficient quantities of carotene, ascobic acid, iron, methionine and cystine (Makkar and Becker, 1996). Apart from these nutritional constituents, Moringa leaves are known to contain phenols, anti-nutritional factors such as tannins, saponins, phytate and oxalate (Gupta et al., 1989). Few studies have showed the effects of Moringa oleifera leaves on the improving of ruminants farming (Gadzirayi et al., 2012) and poultry performances (Banjo, 2012; Portugaliza and Fernandez, 2011; Kakengi et al., 2007). However, chemical compositions of plants may be affected by climatic, seasonal and processing methods (Dei et al., 2007). Therefore, the aim of this study was to determine the level of some anti-nutritional factors of Moringa oleifera leaves and to investigate the effects of the incorporation of different levels of Moringa oleifera leaves in chicken feed on production performance.

**MATERIALS AND METHODS**

**Experimental design: Moringa oleifera leaves** were dried under air conditioning system. Samples of the leaves were analyzed for flavonoid, phenols, polysaccharides and tannins levels determination. In addition, dried leaves were pulverized into powder and incorporated into chicken basal starter diet at different rates e.g., 1 and 2%. So, a total of 815 day-old broiler chicks (Ross) were used. The chicks were divided in to 3 groups, with 4 replications of 50 chicks for each group, fed with basal diet (control, M0), diet with 1% (M1) or 2% (M2) of Moringa oleifera leaves. Crude fibre, Crude protein and metabolizable energy levels of different diets are shown in Table 1. For each feeding treatment, feed and water were provided *ad libitum* during 4 weeks. Prior to divide the birds in different feed treatments, the chicks were weighed and sample of 15 chicks were slaughtered to determine initial weights of liver, heart, pancreas, spleen, rate and thymus. Then during 4 weeks, chick weights as well as organ weights from sample of 10 birds per treatment were recorded weekly.

Also, feed intake and feed conversion ratio were determined for each treatment. Chick body weights and organ absolute weights were used to determine organ relative weights as: 100 x (organ absolute weight/chick body weight) for individual chick. Also, feed intake and feed conversion ratio were determined for each treatment.

**Phytochemical analysis**

**Chemical reagents:** Sodium carbonate is purchased from Sigma chemical company, acid gallic and flavonoid's phenol reagent from Sigma Aldrich company, aluminum chloride, sodium acetate, rutin hydrate 95%, aqueous solution of phenol, sulphuric acid and ethanol are used during the trial.

**Plant material and extraction:** Moringa oleifera leaves were collected in Akoomapé (Vo, Togo) at 25 kilometers from Lomé. They were identified by the department of Botany where a specimen of the plant was deposited in its herbarium. Prior to the determination of phytochemical substances, the leaves were pulserized into powder. The powder (445g) was soaked in ethanol-water (5 litters of ethanol+5 litters of distilled water) for 48 h. The mixing obtained was homogenized, filtered and evaporated at 40°C in Rotavapor R-210 and Heating bath B-491 (Debale, 2002). The hydro-alcoholic extract obtained weighed 52g and was refrigerated at -20°C.

**Determination of total flavonoids:** The determination of total flavonoids is based on the capacity of flavonoids to form, together with aluminium chloride, chelates of aluminium (Kosalec et al., 2004). Rutin, aluminium chloride and sodium acetate are used. To 2 ml of aluminium chloride (20 mg/ml) and 6 ml of sodium acetate (50 mg/ml), are added 2 ml of alcoholic extract (1mg/ml) or standard solution of rutin. All the determinations are carried out in triplicate. After 150 min. of incubation in ambient temperature, the Optic Density (OD) is obtained at 440 nm.

**Determination of total phenols and tannin:** Reagents such as folin ciocalteu, polyvinylpolypyrolidone (PVP), gallic acid, sodium carbonate are used for total phenols and tannin determination. The goal of this method is to obtain total phenols after fixing of tannin by polyvinylpolypyrolidone (PVP). In tubes containing PVP and ethanol, 500 µl of extract are transferred. The mixing obtained is incubated during 30 min. on icer and centrifuged. On 200 µl of the solution obtained after centrifugation or 200 µl of extract solution or 200 µl of solutions of gallic acid (200, 150, 100, 50, 25 and 0 µG/ml) are added 200 µl of Folin-cioalcteu 10%. After 15 min. of incubation at ambient temperature, 800 µl of solution of sodium carbonate (700 mM) are added. The optic density is obtained at 735 nm.
Determination of polysaccharides: Aqueous solution of phenol 5%, sulphuric acid and glucose as standard are used in this assay (Dubois et al., 1956). In a series of tubes containing 200 µL of distilled water or extract solution or glucose (50, 100, 150 and 200 µg/ml) was added 200 µL of aqueous solution of phenol 5% and 1 ml of sulphuric acid solution. All the samples are duplicated. After homogenization, the mixing obtained was introduced in bain-marie at 100°C during 5 min. and cooled in obscurity during 30 min. The optical density is obtained at 480 nm.

The quantities of these different components are expressed in % of dry matter.

Statistical analysis: The data obtained were expressed processed with the statistical software package GraphPad. Generalized linear regression was used to analyze the effects experimental diets on chick and organ weights, feed intake and body weight gain. When the means of the general model were statistically different, then the means were further compared using Turkey’s test. For all analyses, P-value of 0.05 was retained as the degree of significance.

RESULTS
Concentrations of total flavonoids, total phenols, tannin and polysaccharides in Moringa oleifera leaves: Phytochemical screening of the extract indicated the presence of flavonoids, phenols, tannins and polysaccharides. The composition of Moringa oleifera dried leaves in these biological constituents is summarized in Table 2 where are expressed the concentrations of Moringa leaves in some nutritional components like total flavonoids (0.2%), total phenols (4.2%), tannin (2.38%) and polysaccharides (21.1%).

Effect of Moringa leaves on production performance: Chick weights up to 28 d-old in relation to feeding treatments are shown in Table 3. Overall, chick weights increased significantly with age (p<0.05). Until 14 d-old, chick weights were comparable between treatments. From 21-28 days of age, chicks of M1 and M2 groups were not different but were heavier than those of M0 group (p<0.05).

Mean values of feed intake, body weight gain and feed conversion ratio are indicated in Table 4. Feed intakes were comparable between control, M1 and M2 groups. But, daily body weight gains were lower (p<0.05) in M0 group than those of M1 and M2 groups which were similar. On contrary, feed conversion ratios were in the following order: M0<M1 = M2 (p<0.05).

Effects of Moringa leave on relative organ weights: Table 5 shows relative organ weights of liver, pancreas, spleen, bursa and thymus according to feed treatments. For each organ, relative organ weights of group M0 were smaller than those of groups M1 and M2 (p<0.05). Between M1 and M2, relative weights of all organs were not significantly different.

DISCUSSION
This study has revealed the presence of 0.2% of total flavonoid and confirmed the presence of total phenol, tannin and polysaccharides at high quantity compared to the results of Gupta et al. (1989) and Foidl et al. (2001) who pointed out that the quantities of these chemical groups are respectively 3.4, 1.4 and 19.1%. These differences may be due to climatic, seasonal and processing methods effects as showed by Dei et al. (2007). Feed intakes obtained were lower than the standard according to Ross and Enriques (1989). It can be partly due to climatic conditions of rearing as showed by Teteh et al. (2010). The similarity between feed intakes is the line of reports of Sanchez et al. (2005) who pointed out that Moringa did not have any toxic effect or contain any factors limiting intake in opposite of nutrient absorption. Concerning growth performances, body weight, body weight gain, feed conversion and relative
organ weight are influenced differently by the rate of incorporation of Moringa leaves in the basal diet. The three groups have showed a regular growth from the beginning to the 28th day although the weights at this age were lower than those indicated by Ross and Enriquez (1969). The better growth performance of groups M1 and M2 compared to M0 was probably due to the high protein content of Moringa oleifera leaves as claimed by Banjo (2012). Indeed, these results are the line of reports of Banjo (2012) who showed that at 6 weeks of age broiler chicks fed with diets containing 1% and 2% of Moringa leaves showed higher weight gain than those fed with diet containing 0% of Moringa leaves. In addition, the high digestibility of Moringa leaves (Becker, 1995) could improve absorption of nutrients. In this context, an experiment conducted at Poultry Science Laboratory of University of Loré (none published), showed that the inclusion of 1 and 2% of Moringa leaves in chicks diet resulted in longer and heavier duodenum, jejunum and ileal than chicks fed with basal diet. This should be equivalent to a very important villus in the intestinal mucous (Soraya et al., 2009) and therefore an important digestive absorption surface (Samanya and Yamauchi, 2002). This effect of Moringa leaves leads to higher daily weight gain and lower feed conversion of M1 and M2 groups compared to M0 group. Also, the significant difference about relative weights of liver and pancreas point out that Moringa leaves improve the metabolism and the digestion of macronutrients contained in diets 2 and 3 (Picard et al., 1999). High relative weights of spleen, bursa and thymus in groups M1 and M2 compared to group M0 can be explained, according to Ruiz-Feria and Abdulkalykova (2009), by an important proliferation of lymphocytes T and B. This important production of the immune cells may be due to antioxidant activities of some components of Moringa leaves like vitamins C, E (Rocha et al., 2010) and phenols (Diallo et al., 2009) especially flavonoids (Cailliet et al., 2006) and to the capacity of plants polysaccharides to modulate the immune system (Dong et al., 2007).

Concerning body weight, daily weight gain and relative organ weights, from d-old to 28th day, there is no significant difference between M1 and M2 in spite of the incorporation of 2% of Moringa leaves in diet 3. This similarity can be explained by the presence in high quantity in diet 3 of some anti-nutritive factors such as fibres (Banjo, 2012; Kakengi et al., 2007), tannins and especially saponins (Gupta et al., 1989). Indeed, an experiment conducted in the Poultry Science Laboratory of the University of Loré (unpublished) showed that blood of chicks of group M2 has contained a significant lower level of triglyceride than blood of group M1. This low level of triglyceride was attributed to hypolipidaemia properties of saponins by Dong et al. (2007) and Mehta et al. (2003). According to Dei et al. (2007), saponins are known to impair the digestion and limit the capacity of intestinal mucous to absorb nutrients especially lipids those are one of the important sources of energy. Then, these anti-nutritive factors may start to exert their effects from 2% of rate of incorporation in opposite to Banjo (2012) who showed that these effects start from the rate of 3% because of the high content of fibres only.

It can be concluded that Moringa oleifera leaves incorporated in poultry feed at the place of antibiotic growth promoters can improve growth performance when used at 1 and 2%.

REFERENCES


