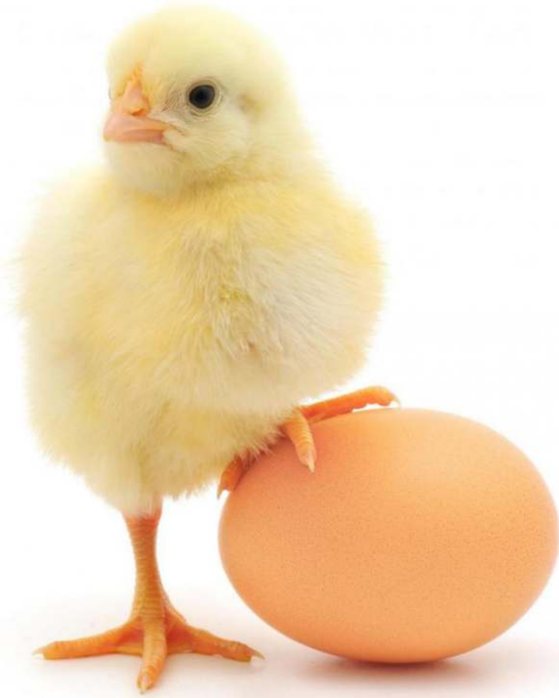
ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 12 (7): 401-405, 2013
ISSN 1682-8356

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

A. Teteh[1], E. Lawson[2], K. Tona[1], E. Decuypere[3] and M. Gbeassor[1]
[1]Laboratory of Poultry Sciences, Faculty of Sciences, University of Lomé, B.P. 1515, Lomé, Togo
[2]Laboratory of Pharmacology of Natural Substances, Faculty of Sciences, University of Lomé, B.P. 1515, Lomé, Togo
[3]Laboratory for Physiology, Immunology and Genetics of Domestics Animals, Department of Biosystems, K.U. Leuven, Belgium

Abstract: After the ban in 2006 of the use of antibiotic growth promoter, the search of 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 Akoumapé (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 (2.38%), 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 (Griggs and Jacob, 2005). A review of available literature shows that *Leucena leucocephala*, *Gliricidia 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 it 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

Corresponding Author: A. Teteh, Laboratory of Poultry Science, University of Lome, B.P. 1515 Lome, Togo

protein, 12 MJ/Kg of metabolizable energy and to possess 79.7% of digestibility (Becker, 1995). It also contents sufficient quantities of carotene, ascorbic 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 615 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.

Table 1: Gross compositions of experimental diets (%)

	Groups		
Feed stuffs	M0	M1	M2
Maize	56	55.44	54.88
Wheat bran	9	8.91	8.82
Soya seed	22	21.78	21.56
Fish meal	9	8.91	8.82
Concentrate	3	2.97	2.94
Oyster shell	0.75	0.74	0.73
Salt	0.25	0.247	0.245
Moringa leaves (%)	0	1	2
Total	100	100	100
Calculated analysis			
CP (%)	20.65	20.67	20.70
ME (Kcal/Kg)	3000	2999.39	2998.78
CF (%)	4.89	5.05	5.21

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 folin-ciocalten's phenol reagent from Sigma Aldrich company, aluminium 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 Akoumapé (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 phyto-chemical substances, the leaves were pulverised 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 flavonoïds: The determination of total flavonoïds is based on the capacity of flavonoïds 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, polyvinylpolypyrolydone (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 polyvinylpolypyrolydone (PVP). In tubes containing PVP and ethanol, 500 µL of extract are transferred. The mixing obtained is incubated during 30 min. on icicle 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-ciocalteu 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 optic 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 flavonoïds, total phenols, tannin and polysaccharides in *Moringa oleifera* leaves: Phytochemical screening of the extract indicated the presence of flavonoïds, 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 flavonoïds (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.

Table 2: Levels of chemical groups contained in hydro-alcoholic extract of *Moringa* leaves (% of dry matter)

Chemical groups	Quantity (% of dry matter)
Total phenols	4.2
Total flavonoids	0.2
Tannins	2.38
Polysaccharides	21.1

Table 3: Chick weights according to developmental stage and feed treatment

	Diet treatments		
DS	M0	M1	M2
d-old	50.46±0.41[a]	50.62±0.60[a]	50.22±1.08[a]
7 d-old	110.39±3.84[a]	109.37±3.47[a]	109.56±2.90[a]
14 d-old	235.49±8.64[a]	239.04±7.50[a]	239.47±7.31[a]
21 d-old	410.54±14.41[b]	440.95±13.41[a]	438.65±12.34[a]
28 d-old	681.14±14.00[b]	789.28±10.75[a]	772.37±17.45[a]

[a,b]Within row, data sharing no common letter are different (p<0.05). DS: Developmental stage

Table 4: Daily feed intake, daily weight gain and feed conversion according to feed treatment

	M0	M1	M2
Daily Feed intake (g)	51.46±0.61[a]	51.62±0.6[a]	51.3±0.4[a]
Daily weight gain (g)	22.5±0.41[b]	26.4±0.36[a]	25.8±1.15[a]
Feed conversion ratio	2.28±0.15[a]	1,95±0.01[b]	1,98±0.06[b]

[a,b]Within row, data sharing no common letter are different (*p*<0.05)

Table 5: Relative organ weights according to feed treatment

	Groups		
Organs	M0	M1	M2
Liver	2.66±0.17[b]	3.44±0.26[a]	3.30±0.26[a]
Pancreas	0.27±0.055[b]	0.35±0.07[a]	0.33±0.058[a]
Spleen	0.096±0.019[b]	0.115±0.021[a]	0.117±0.021[a]
Bursa	0.136±0.030[b]	0.226±0.051[a]	0.238±0.047[a]
Thymus	0.073±0.013[b]	0.093±0.045[a]	0.109±0.07[a]

[a,b]Within row, data sharing no common letter are different (p<0.05)

DISCUSSION

This study has revealed the presence of 0.2% of total flavonoïd 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 Enriquez (1969). 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 Lomé (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 muqueous (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 Abdukalykova (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 flavonoïds (Caillet *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 Lomé (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

Banjo, O.S., 2012. Growth and performance as affected by inclusion of *Moringa oleifera* leaf meal in broiler chicks diet. J. Biol. Agric. Healthcare, 2: 35-38.

Becker, K., 1995. Studies on utilization of *Moringa oleifera* leaves as animal feed. Institute for Animal Production in the Tropics and Subtropics vol. 480. University of Hohenheim Stuttgart, p.15.

Caillet, S., S. Salmieri and M. Lacroix, 2006. Evaluation of free radical scavenging properties of commercial grape phenol extracts by a fast colorimetric method. Food Chem., 95: 1-8.

Debale, A., 2002: Manual for phytochemical screening of medicinal plants. Department of drug research. Ethiopian Health and Nutrition Research Institute (EHNRI), Ethiopia, pp: 15-24.

Dei, H.K., S.P. Rose and A.M. Mackenzie, 2007. Shea nut (*Vitellaria paradoxa*) meal as a feed ingredient for poultry. World's Poult. Sci. J., 63: 611-624.

Diallo, A., K. Eklu-Gadegbeku, T. Mobio, S. Moukha, A. Agbonon, K Aklikokou, E.E. Creppy and M. Gbeassor, 2009: Protective effect of *Moringa oleifera* Lam. and *Lannea kerstingli* extracts against Cadnum and Ethanol-induced lipid peroxidation. J. Pharmacol. Toxicol., 4: 160-166.

D'Mello, J.P.F., T. Acamovic and A.G. Walker, 1987: Evaluation of leucaena leaf meal for broiler growth and pigmentation. J. Trop. Agric. 64: 33-35.

Dong, X.F., W.W. Gao, J.M. Tong, H.Q. Jia, R.N. Sa and Q. Zhang, 2007: Effects of Polysavone (Alfalfa Extract) on abdominal fat deposition and immunity in broiler chickens. Poult. Sci., 86: 1955-1959.

Dubois, M., K. Gilles, J. Hamilton, P. Rebers and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem., 28: 350-356.

Foidl, N., H.P.S. Makkar and K. Becker, 2001: The potential of *Moringa oleifera* for agricultural and industrial uses, the miracle tree: The multi uses of *Moringa* Ed. Lowell J. Fugile, CTA, Wageningen, The Netherlands, pp: 45-76.

Gadzirayi, C.T., B. Masamha, J.F. Mupangwa and S. Washaya, 2012. Performance of broiler chickens fed on mature *Moringa oleifera* leaf meal as protein supplement to soyabean meal. Int. J. Poult. Sci,. 11: 5-10.

Gould, I.M., 2008. Antibiotic policies to control hospital-acquired infection. J. Antimicrobial Chemotherapy, 61: 763-765.

Griggs, J.P. and J.P. Jacob, 2005. Alternatives to antibiotics for Organic Poultry Production. J. Appl. Poult. Res., 14: 750-756.

Gupta, K.B., G.K. Barat, D.S. Wagle and H.K.L. Chawla, 1989. Nutrient contents and antinutritional factors in conventional and non-conventional leafy vegetables. Food Chem., 31: 105-106.

Kakengi, A.M.V., J.T. Kaijage, S.V. Sarwatt, S.K. Mutayoba, M.N. Shem and T. Fujihara, 2007. Effect of *Moringa oleifera* leaf meal as substitute for sunflower seed meal on performance of laying hens in Tanzania. Livestock Res. for Rural Development 19, pp: 12.

Kosalec, I., M. Bakmaz, S. Pepeliniak and S. Vladmir-Knezevic, 2004. Quantitative analysis of the flavonoids in raw propolis from northern Croatia. Acta Pharmacol., 54: 65-72.

Makkar, R., H.P.S. and K. Becker, 1996. Nutritional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. Anim. Feed Sci. Technol., 63: 211-228.

Mehta, L.K., R. Balaraman, A.H. Amin, P.A. Bafna and O.D. Gulati, 2003. Effect of fruits of *Moringa oleifera* on lipid profile of normal and hypercholesterolaemic rabbits. J. Ethnopharmacol., 86: 191-195.

Picard, M., P. Siegel, C. Leterrier and P.A. Geraert, 1999. Diluted starter diet, growth performance and digestive tract development of fast-and slow-growing broilers. J. Appl. Poult. Res., 8: 122-131.

Portugaliza, H.P. and T.J. Fernandez Jr., 2011. Growth performance of Cobb broilers given varying concentrations of Malunggay (*Moringa oleifera* Lam.) aqueous leaf extract J. Anim. Feed Res., 2: 465-469.

Rocha, J.S.R., L.J.C. Lara, N.C. Baiao, R.J.C. Vasconcelos, V.M. Barbosa, M.A. Pompeu and M.N.S. Fernandes, 2010. Antioxidant properties of vitamins in nutrition of broiler breeders and laying hens. World's Poult. Sci. J., 66: 261-270.

Ross, E. and F.Q. Enriquez, 1969. The nutritive value of cassava leaf meal. Poult. Sci., 48: 846-853.

Ruiz-Feria, C.A. and S.T. Abdukalykova, 2009. Arginine and vitamin E improve the antibody responses to Infections Bursal Disease Virus (IBDV) and sheep red blood cells in broiler chickens. Br. Poult Sci., 50: 291-297.

Samanya, M. and K.E. Yamauchi, 2002. Histological alterations of intestinal villi in chickens fed dried *Bacillus Subtilis var. Natio*. Comp. Biochem. Physiol. Part A, 133: 95-104.

Sanchez, N.R., E. Sporndly and I. Ledin, 2005. Effect of different levels of foliage of *Moringa oleifera* to creole dairy cows on intake, digestibility, milk production and composition. Livest., Sci., 2810: 8.

Soraya, T., H. Nabila, B. Larbi, S. Linda, K. Rachid, B. Hocine, K. Djamel, A. Karim and A.B. Hacina, 2009. Evaluation de l'efficacité du probiotique Pediococcus Acidilactici sur les performances de croissance, la morphométrie et la flore lactobacillaire de l'intestin du poulet de chair. Eur. J. Sci. Res., 38: 119-128.

Suarez, M., M. Haenni, S. Canarelli, F. Fisch, P. Chodanowski, C. Servis, O. Michelin, R. Frietag, P. Moreillon and N. Mermod, 2005. Structure-Function characterization and optimization of a plant-derived antibacterial peptide. Antibacterial Agents Chemotherapy, 49: 3847-3857.

Teteh, A., K. Tona, K. Aklikokou, M. Gbeassor, J. Buyse and E. Decuypere, 2010. Effects of low-protein or high energy levels diets on layer-type chick juvenile performance. Int. J. Poult. Sci., 9: 1156-1160.

WHO, 2008: Antimicrobial resistance. World Health Organization Media Centre. Fact sheet N°194. http://www.who.int/mediacentre/factsheets/fs194/en/. Accessed on 9th April, 2008.