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Water supplementation of *Moringa oleifera* as a substitute for antibiotics on performance and blood parameters of broiler chickens

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Abstract

There is a serious public health concern of the use of antibiotics sub-therapeutically and/or as growth promoter in poultry feed and water. Therefore, there is an urgent need for alternatives. *Moringa oleifera* is a phytobiotic, which has been reported to possess antimicrobial and immuno-modulatory properties. This study aimed to investigate the effects of aqueous *M. oleifera* leaf extract (MOLE) supplementation on growth performance and hematological indices of broiler chickens. The 36 day-old broiler chicks (Cobb 500) were randomly divided into three dietary groups (group A, B and C) having 12 birds in each. The group A was considered as negative control which had received the basal diet and fresh drinking water. The group B was fed with basal diet and antibiotics (Ciprofloxacin) plus Vitamin B-complex mixed drinking water(Positive control). The group C was fed with basal diet and drinking water supplemented with 1% MOLE. Body weight, feed intake and feed conversion ratios of the birds were recorded on weekly basis. On day 35th of the feeding trail, blood samples from five birds per replicate were used for the study of hematological indices. Birds under Group C attained intermediate body weight (1892 g) and best FCR (1.48) those were comparable with that of Group B but better ($p<0.05$) than those of Group A. There was a trend of decrease ($p>0.05$) internal organs weight in Group C compared to other groups. However, zero mortality rate and similar hematological indices were observed in the birds across the treatments. Here, the results suggested that, the aqueous MOLE can be included in the drinking water of broiler chicken for reduced feed intake and improved feed conversion efficiency and it can be considered as an alternate to synthetic antibiotics as growth promoter to fight the emergence of antibiotic resistance phenomena in poultry industry.

Introduction

Emergence of antibiotic resistant pathogens due to subtherapeutic use of antibiotic as growth promoters (AGPs) carriages a serious hazard for human health. European Union (EU) already has banned the use of antibiotics as feed additives in poultry (EC Regulation No. 1831/2003) as they pose a threat to human health by development of resistant pathogens (Sarker *et al.*, 2018).This has headed the scientists to look for feed alternatives, which can serve more than one purpose. One such resource is a group of feed additives termed as phytogenic products or phytobiotics, which include whole plants or their parts (Khan *et al.*, 2017). One such plant, *Moringa oleifera*, belongs to the family of Moringaceae and is considered indigenous in south Asia including Bangladesh (Zvinorova *et al.*, 2015). The dried, ground leaves of *M. oleifera* contain 25.1% crude proteins, 5.4% lipids, 11.5% ash, 21.9% NDF (neutral detergent fibre) and 11.4% ADF (acidic detergent fibre), 44.4% carbohydrates (Teixeira *et al.*, 2014). Vitamins A, C and E as well as their provitamins present in *M. oleifera* leaves are known to sequester free radicals and may have immunoprotective effects. Additionally, the leaves are reported to contain high amounts of total phenols, flavonoids, carotenoids and are a good source of potassium and magnesium (Teixeira *et al.*, 2014). The aqueous extract of *Moringa oleifera* leaf (MOLE) has antinflammatory, antioxidant, antiulcerous, antihyperlipidaemic and anticancereous properties (Coppin, 2008). Antifungal and broad-spectrum antibacterial activities of *Moringa oleifera* fruit extracts have also been reported (Bukar *et al.*, 2010). Toxicity studies have showed that *M. oleifera* leaf extract has no reported significant adverse effects in humans, rats, rabbits or poultry (Ashong and Brown, 2011; Stohs and Hartman, 2015). Rich nutrient profile of *M. oleifera* suggests potential growth promoter and immunomodulatory effects. Partially these effects have been measured in poultry with variable results, but the information on modulation of gut associated immune components is lacking. It is hypothesized that *M. oleifera* promotes growth performance by influencing gut architectural integrity and gut-associated immune components. Therefore, this study aims to investigate the effects of dietary supplementation of 1% MOLE against conventional antibiotics on growth performance and blood parameters of broiler chickens.

Materials and Methods

Study location

Collected birds were reared in iron wired cage in an environmentally controlled experimental shed of the Department of Pharmacology, Bangladesh Agricultural University, Mymensingh, Bangladesh, located at 24°43´N 90°25´E, and elevation 45 feet.

Test ingredients

To prepare MOLE, fresh green and undamaged mature *Moringa oleifera* leaves were collected and dried in shady area with no direct sunlight exposure to avoid leaching, with constant turning over to avert fungal growth. After drying, the leaves were crushed to a fine powder. Extraction of *Moringa oleifera* leaves was performed by Soxlhet extraction method according to Rajput *et al.* (2017).

Bird's management

A total of 36 day-old chicks (Cobb 500) were randomly divided into three groups (12 birds in each group) in a Completely Randomized Design (CRD). Treatment randomization was ensured by assigning a continuous sequence to the replicates of different groups. The temperature and relative humidity (RH) of experimental house was maintained at 35±1° C and 70±5%, respectively, during the first week, and then, temperature was decreased by 3°C per week until it reached 25±1°C, with RH65±5% and maintained the same till the end of trial, that is day 35. Birds in the negative control group (Group A) were fed a basal diet only. The positive control group (Group B) was fed basal diet and antibiotic (Ciprofloxacin; Renaflox®, Renata Animal Health, Dhaka, Bangladesh), Vitamin B-complex (B-comvit®, Square agrovet and pesticide, Dhaka, Bangladesh) supplied as pharmaceuticals recommended dose. Birds in experimental group (Group C) were fed the same diet supplemented with MOLE at1% mixed with drinking water. Daily recorded feed consumption and average body weight estimated on weekly basis were used to calculate feed conversion ratio (FCR).

Performance parameters

During the experiment many performance parameters were estimated including weekly body weight to the nearest gram using sensitive scale, weekly body weight gain, feed intake per bird per week, water intake per bird per week, mortality per treatment per week and feed conversion which was estimated according to Lambert *et al.* (1936).

Blood collection

At the end of the experiment, 3 ml of blood was collected from 5 randomly selected birds from each replicate *via* the wing vein into test tubes. Blood samples for haematological analysis were collected into sterilized test tubes containing ethylene diamine tetra acetic acid (EDTA) as anti-coagulant while those used for serum biochemical analysis were collected into tubes without EDTA and centrifuged before analysis. Packed Cell Volume (PCV), Total Erythrocyte Count (TEC) and Haemoglobin concentrations (Hb) were measured as described by Lamberg and Rothstein (1977).

Data collection

The following data were collected and determined over a period of five weeks.

Feed intake (g)

The feed given to the birds in each treatment was weighed daily and the left over feed was also weighed. The daily feed consumed was determined by finding the differences between the left over and the initial quantity of feed given. The weekly records of average feed consumed were obtained for each of the replicate by dividing the total quantity of feed consumed by the total number of chickens in each replicate.

Water intake (ml)

The drinking water given to the birds in each treatment was measured daily and the left over water were also measured. Water intakes were determined by calculating the difference between the left over and the initial quantity of water given.

Body weight gain (g)

The body weight gains for each week were determined by subtracting the previous week's body weight from the current week's body weight. The initial weights of the birds were taken at the commencement of the study. The record obtained was used to calculate the average body weight gain per replicate (the weight for each replicate was added per replicate and then divided by the number of birds in each replicate).

Feed Conversion Ratio (FCR)

The FCR was determined from the average weight gained and average feed consumed by the birds in each treatment.

Feed conversion ratio (FCR) = average feed intake (g) / average body weight gain (g)

Chemical analysis

Proximate composition of the *Moringa oleifera* leaf extract was determined using the procedures of AOAC (2000). The main results were illustrated in Table 1.

Statistical analysis

Data collected were subjected to one way analysis of variance (ANOVA) based on the Completely Randomized Design model, using Statistical Analysis System (SPSS IBM 20). Where differences occurred at 5 % ($p<0.05$), they were separated using Duncan's Multiple Range Test.

Table 1. Proximate composition (%) of *Moringa oleifera* leaf extract

Parameters	DM	CP	EE	CF	NFE	Ash
Composition (%)	92.53	24.28	5.87	19.07	56.32	10.05

DM: Dry matter; CP: Crude protein; EE: Ether extract; CF: Crude fibre; NFE: Nitrogen free extracts

Results and Discussion

Productive performance

Body weight gain was influenced significantly for the aqueous supplementation of *Moringa oleifera* leaf extract (MOLE). Highest body weight gain was observed in group B (1920 gm.), followed by group C (1892 gm.) and lowest gain was observed in group A (1640 g). Weekly weight gain details were shown in Table 2.

Table 2. Weekly live weights (g)

Group	Live weight gains (Mean±SEM)					
	Initial	1st week	2nd week	3rd week	4th week	5th week
Group A	41.36 ±0.67	95.60 ±1.36	304.60 ± 9.11	867.00 ±29.65	1359.00 ± 45.99	1640.00[b] ±74.83
Group B	43.43 ±0.76	97.56 ±0.92	335.00 ±7.23	918.00 ±19.91	1423.20 ± 37.47	1920.00[a] ±33.91
Group C	43.92 ±0.81	98.10 ±0.64	325.80 ± 4.27	895.00 ±36.40	1362.40 ± 26.65	1892.00[a] ±30.23

[abc] Different lettering in the same column indicates significant variation at the 5% level.

Moringa oleifera leaves due to their high nutritional, antimicrobial and immunomodulatory properties have been reported as a potential dietary supplement for poultry in emerging scientific articles (Abbas, 2013; Akhouri *et al.,* 2013; Paguia *et al.,* 2014). However, consistencies in the findings reported in literature as well as preferred use of MOLE as a replacement diet in poultry research warrants dose-dependent trials based on small increments of MOLE within safe limits. Similar weight gain was observed by Talha *et al.* (2012); Gadzirayi (2014) and Gakuya *et al.* (2014).

Feed Conversion Ratio (FCR)

MOLE of water supplementation at 1% decreased FCR than negative control where in positive control lowest FCR was observed among the groups. However the difference was significant at 5% level. In details of weight gain and feed conversion were shown in Table 3. The present findings are in accordance with Safa (2012) and Kout *et al.* (2015) who also observed significantly higher body weights on diets containing different levels of *M. oleifera* leaf extract. Nkukwana *et al.* (2012) also found that birds supplemented with *M. oleifera* leaf extract had higher FCR than the birds fed the control diets. *M. Oleifera* plant was reported to contain various amino acids, a highly potent anti-inflammatory (Ezeamuzle *et al.,* 1996), and hepatoprotective properties (Pari and kumar, 2002). The leaves of the tree have been reported to have an antioxidant activity due to the higher number of polyphenols (Moyo *et at.,* 2012).

The present results are in agreement with Kout *et al*. (2015) who recorded best feed conversion ratio in birds fed on 0.2% MOLM. Sherief *et al.* (2012) also reported significantly better feed conversion efficiency in Moringa leaf powder supplemented groups. The results of the present findings are not in agreement with the reports of Divya *et al.* (2014) who reported that no significant difference was observed in FCR of the broiler chickens basal diet with four levels of *Moringa* leaves powder which might be due to high level of *Moringa oleifera* leaf powder in diets.

Table 3. Final weight gain, feed intake and FCR

Parameters	Experimental groups			SEM
	A	B	C	
1[st]week body weight (g)	95.60[a]	97.56[b]	98.10[b]	0.76
Final body weight (g)	1640[a]	1920[b]	1892[b]	89.03
Feed intake	3030	2990	2800	70.95
FCR	1.85[a]	1.56[b]	1.48[c]	

[abc] Means in the same row with different superscripts differ significantly ($p<0.05$);
SEM = Standard error of means;
FCR = Feed conversion ratio

Feed intake and mortality

The mean weekly feed consumption was highest in group A, followed by group B and lowest was observed in group C. Details weekly feed intake was shown in Table 4. No mortality was found throughout the experimental period. The result revealed that there was significant difference between the treatments in average feed consumption of broilers in comparatively with others group.

Table 4. Average feed intake (g) per week (F/W) of broilers

Group	Feed intake per week (F/W)					
	1[st] week	2[nd] week	3[rd] week	4[th] week	5[th] week	Total
A	102	436	610	982	900	3030
B	101	419	710	860	900	2990
C	101	399	710	830	760	2800

The present result agreed with Kout *et al.* (2015) who observed that different levels of *Moringa oleifera* leaf meal (0.0, 0.2, 0.4 and 0.6%) showed lowest feed consumption at 0.2% Moringa oleifera leaf meal compared to other groups. Ochi *et al.* (2015) reported that during finisher and the whole period supplying broiler chicks' diet with 1% *M. oleifera* seed powder resulted in significant decreased in feed consumption. However, Paguia *et al.* (2014) studied the influence of *Moringa oleifera* leaf meal basal diet, 0.1%, 0.2%, 0.3%, 0.4% on growth performance of broilers and was found to have no effect on average cumulative feed consumption. These variations observed may be due to different levels used.

Inclusion of *Moringa oleifera* leaf extract in broiler diet with different level did not showed any adverse effect on mortality of broiler under study. Whereas, Karthivashan *et al.* (2015) reported approximately 2% mortality was observed in all groups and there were no significant differences in all treatment groups.

Internal organ weight

Internal organs weight did not differ significantly among the groups. Weight of liver, gizzard, heart, spleen and pancreas of different dietary treatment groups were

shown in Fig. 1. In the MOLE treated group internal organs are supposed to decrees its weight gain. Onunkwo *et al.* (2015) evaluated the effects of *Moringa oleifera* leaf meal on growth performance and carcass characteristics of boiler chicks. The different graded levels of MOLE were studied in broilers. There was significant difference in organ weights (wings, shank, drumsticks, kidney, liver, and gizzard) and some cut parts between the experimental and control groups. Similar findings also observed by Talha *et al.* (2012) and Divya *et al.* (2014).

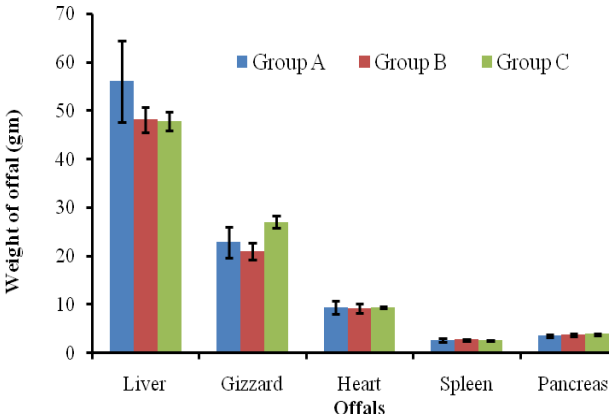

Fig. 1. Weights of liver, gizzard, heart, spleen and pancreas in different dietary groups (Mean±SEM)

Hematological indices

Haematological characteristics of livestock have been observed as factors determining the response of livestock to the diet they are fed (Madubuike *et al.*, 2006). The haematological indices of birds fed the experimental diets are shown in Table 5. The results showed that there were no significant differences across the treatments in all the haematological indices measured. This finding is similar to the results of Ewuola *et al.* (2012) who reported that there was no significant difference across the treatments for growing rabbits fed graded levels of *Moringa oleifera* leaf meal. The values obtained in this study observed within the normal range for healthy chickens as described by Animashahun *et al.* (2006). The results of hematological parameters indicated that the dietary treatments were nutritionally adequate for the birds.

Table 5. Haematological parameters of broilers

Group	Hematological parameters		
	RBC (mil/cmm)	Hb (gm)	PCV (%)
A	2.90±0.06	7.44±0.12	25.20±1.53
B	2.81±0.03	7.36±0.09	25.80±0.37
C	2.90±0.05	7.60±0.07	28.00±0.45

Conclusion

The results of the study showed that most of all the parameters measured in birds fed diets containing *Moringa oleifera* leaf extract compared well with those placed on an antibiotic. Although *Moringa oleifera* leaf extract is generally considered a protein source in poultry nutrition, it could be a promising natural antimicrobial agent for controlling pathogenic bacteria in livestock and poultry production if its antimicrobial potential is further investigated and harnessed.

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