Effect of aqueous herbal extracts on growth, plasma metabolites and meat characteristics of broiler

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Abstract

The experiment was carried out for 4 weeks to find out the efficacy of herbal extracts supplementation in drinking water on production performance, plasma metabolites, carcass characteristics, and fat deposition of commercial broilers. A total of 200 straight run day-old chicks (Cobb-500) were divided into five treatment groups; T_0=Basal diet, T_1= Basal diet+10 ppm neem leaves extract, T_2= Basal diet+10 ppm garlic leaves extract, T_3= Basal diet+10 ppm plantain leaves extract, T_4= Basal diet+10 ppm antibiotic growth promoter (AGP) each with four replications having 10 birds per replicate. All the herbal extracts and AGP were supplemented in drinking water. Ration in the form of crumble was offered to the birds ad libitum having ME=3060 kcal/kg and CP=22.5%. Body weight and feed intake were recorded weekly to calculate body weight gain (BWG) and feed conversion ratio (FCR). A total of 15 birds three from each treatment were sacrificed at the end of feeding trial and dressing parameters were evaluated. Sensory properties of broiler meat were assessed by a panel of experts. Feed intake, BWG and FCR varied (p<0.05) in all treated groups, and the higher BWG and improved FCR were found in AGP group. Triglyceride and very low-density lipoprotein content were lower (p<0.05) in all herbal treated groups than the control and elevated at AGP group. However, increased (p<0.001) dressing percentage and abdominal fat deposition were found in AGP group compared to herbal treated groups and the least value was found in plantain group which also exhibited lower (p<0.001) meat ether extract. Flavor (p=0.001), juiciness (p=0.002), tenderness (p=0.005) and overall acceptability (p<0.001) of broiler meat were significantly higher in herbal group over control and AGP group. The aqueous herbal extract supplementation could be an alternative to antibiotic growth promoter to enhance growth performance, plasma lipid profiles as well as to reduce abdominal fat and meat of either extract.

Key words: *broiler, herbal extracts, blood lipid profile, abdominal fat, sensory test*

Introduction

Feed additives are added to poultry feed for improving nutritive value of ingredients and enhancing broiler performance by increasing growth rate and improving feed conversion efficiency. Chemical feed additives e.g. antibiotic growth promoters (AGP) have long been intensively used in broiler ration to improve productivity. Antibiotic growth promoters are used to help growing animals to utilize their feed efficiently, get maximum benefit from it and allow them to become strong and healthy (Miles *et al.,* 2006). Although the mechanism supporting their action is unclear, it is believed that the antibiotics suppress sensitive populations of bacteria in the intestines. Thomke and Elwinger (1998) hypothesized that cytokines released during the immune response may also stimulate the release of catabolic hormones, which would reduce muscle mass. Therefore, a reduction in gastrointestinal infections would result in the subsequent increase in muscle weight. Whatever the mechanism of action, the result of the use of growth promoters is an improvement in daily growth rates between 1 and 10 percent resulting in meat of a better quality, with less fat and increased protein content.

However, they are notorious for bacterial resistance and their negative impacts on the consumers' health. Thus, use of AGP has been banned in poultry industry. There is evidence that resistance in some human enteric pathogens has arisen because of transfer of resistant bacteria or resistance genes from animals to people via the food chain (Barton, 2000; Roe and Pillai, 2003). Indiscriminate use

of antibiotic growth promoters (AGPs) as sub-therapeutic level in animals and poultry caused consequent appearance of resistance to that particular antibiotic among several pathogenic bacteria (Rerksuppaphol *et al.,* 2003 and Zhao *et al.,* 2003). Cervantes (2004) stated that scientific evidence of antibiotic resistance in food animals is associated with resistance infections in humans. Some scientists believe dependence on and misuse of antibiotics in human medicine is the primary cause of resistance. It has been postulated that re-exposure to previously withdrawn antibiotics will lead to a rebound effect, with very rapid re-emergence of resistance (Salyers and Amabile-Cuevas, 1997). The possible adverse effect of using antibiotics to human health was first reported by Swann (1969). It was mentioned that the probable hazard to human health was from feeding sub-therapeutic levels of antibiotics to food producing animals. Antibiotic resistance genes may be transferred from animal pathogens or commensals to human pathogens (Miles *et al.,* 2006). Gold and Moellering (1996) stated that the majority of human antibiotic resistance problems have connection with use of antibiotics in animals. Several reports (WHO, 1998 and MAFF, 1998) described the link between antibiotic use in animals and the development of resistance in human pathogens.

Considering adverse effect and withdrawal of antibiotic growth promoters by EU, scientists are actively looking for an efficacious alternative to AGPs. Different herbal extracts containing bioactive components have the compatibility. Various herbal products are being used as growth promoters in the poultry rations like garlic leaves (Ahmad, 2005), kalongi (Ihsan, 2003), plantain (Plantago lanceolate L.) which have anti-oxidative activity (Wang *et al.,* 1996) and anti-inflammatory effects (Marchesan *et al.,* 1998), neem leaves rich in nimbin that have free radical scavenging activity. This experiment was designed to find out suitable herbal extract for broiler that might be compatible with AGP.

Materials and Methods

Experimental birds and management

The experiment was conducted in a poultry rearing unit at Shahjalal Animal Nutrition Field Laboratory, Bangladesh Agricultural University, Mymensingh for a period of 28 days. Two

hundred straight run day-old broiler chicks (Cobb-500) were purchased from a commercial hatchery and divided into five treatment groups (T0= Control group, T1= Neem group, T2= Garlic group, T3= Plantain group, T4= Antibiotic growth promoter group) with four replications per treatment having 10 birds in each replication. Continuous lighting was provided throughout the experiment. The brooding temperature was maintained 32^0C in 1st week and thereafter gradually decreased 3^0C in each week until it reached to 21^0C. A completely randomized design was used involving five dietary treatments and four replicates for each. Plantain, garlic and neem was cultivated at Shahjalal Animal Nutrition Field and Quinosol 10% was used as an antibiotic growth promoter. The Ingredients composition and chemical constituents of basal diet are presented in Table 1.

Table 1: Composition and chemical constituents of ration

Ingredients	Amount
Maize (%)	51.50
Rice Polish (%)	2.00
Protein concentrate (%)	6.05
Soybean oil (%)	4.00
Soybean meal (%)	26.50
Mustard oil cake (%)	3.80
Di- calcium phosphate (%)	1.35
Meat and bone meal (%)	4.00
Vit-mineral premix[*] (%)	0.25
Methionine (%)	0.05
Common salt (%)	0.50
Chemical Constituents *(g/100g DM)*	
Dry matter	89.00
Crude protein	22.50
Crude fibre	5.00
Ether extract	4.00
Nitrogen free extract	48.61
Ash	8.39
Calcium	0.84
Phosphorus	0.56

[*]Each kg premix contained: vitamin A palmitate, 6,600 IU; cholecalciferol, 2,200 IU; menadione dimethylpyridine bisulfite, 2.2 mg; riboflavin, 4.4 mg; pantothenic acid, 13 mg; niacin, 40 mg; choline chloride, 500 mg; biotin, 1 mg; vitamin B12, 22 µg; ethoxyquin, 125 mg; iron, 50 mg; copper, 6 mg; zinc, 40 mg; manganese, 60 mg; selenium, 0.2 mg.

Fresh neem leaves, garlic leaves and plantain leaves were harvested at 5 cm above ground level, placed in a polyethylene bag and dried at -30°C by using a vacuum freeze dryer (FRD-50M; Iwaki Glass, Tokyo, Japan) in order to minimize any unwanted changes of bioactive components by oxidation. Then 10 mg dried sample was mixed with 1-liter drinking water to prepare 10 ppm herbal extract and served to birds twice a day. Feed consumption was recorded weekly basis, by recording the feed offered and amounts retained in feeders at end of each week. Individual bird weight was also recorded weekly.

Sample collection and analysis

The proximate components of feeds and meat were analyzed according to AOAC (1990). Blood samples (5 ml each) were collected on day 28 of the feeding trial in a heparinized tube and kept in an ice box until centrifugation. Samples were centrifuged at $10000 \times g$ at 4° C for 10 minutes for plasma separation and plasma was stored at -20° C. Plasma metabolites were analyzed using spectrophotometer (Spectronic, Genesis 5, USA) for determining the total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol against the blank reagent at 510 nm wave length by using different kits.

Statistical Analysis

Analysis of variance was used to test the significance of treatment effects. Differences among treatment means were tested using Tukey's HSD test with a significance level of 0.05 or better. Carcass and visceral organ variables were expressed as percentages of live weights. These analyses were performed using SPSS statistical analysis software (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Growth performance

Supplementation of different herbal extracts with drinking water influenced growth performances of commercial broilers significantly (Table 2). Higher feed intake (3.3-5.2%) was recorded in the treated groups (neem, garlic and plantain) except for antibiotic growth promoter (AGP) over control. Enhanced weight gain in relation to control group (p<0.001) resulted due to inclusion of herbal extracts and AGP. Feed intake in neem, garlic and plantain treated groups was higher (p=0.003) whereas it did not affect the feed conversion ratio (FCR). However, AGP treated group exerted better FCR compared to control which was indifferent with neem and plantain treated groups. Present experiment revealed that supplementation of herbal extracts improved weight gain and feed efficiency of broilers that was compatible with antibiotic growth promoter (AGP). Our result was in agreement with the findings of different researchers who reported that higher weight gain and improved FCR in broilers when drinking water was supplemented with neem leaves extracts (Sarker *et al.,* 2014, Wankar *et al*., 2009), garlic leaves (Meraj *el al.*, 1998), plantain (Chacrabarti *et al*., 2013). The enhanced weight gain might be due to the presence of different bioactive components in herbal extract like allicin in garlic leaves (Martin *et al*., 2016), nimbin in neem leaves (Sultana *et al*., 2007), catalpol, aucubin, acteosides in plantain (Al-Mamun *et al*., 2008). These bioactive components have bactericidal (Gordon and Wareham, 2010), and bacteriostatic properties (Etxeberria *et al*., 2013) and they minimize the adhesion of pathogenic bacteria (E. coli, Clostridium), inhibit the progression of infections in the digestive tract, thus improve nutrient utilization and performance.

Table 2: Production performance of broilers at 28 days at different dietary treatment

Parameters	T_0	T_1	T_2	T_3	T_4	p-value
Initial weight (g)	44±0.6	43±1.1	44±0.9	43±0.5	44±0.7	0.16
Final weight (g)	1095[b]±5	1153[a]±4	1144[a]±4	1170[a]±4	1181[a]±6	<0.001
Weight gain (g)	1051[b]±6	1110[a]±26	1100[a]±12	1127[a]±17	1137[a]±20	<0.001
Feed intake (g)	1783[b]±5	1842[a]±8	1848[a]±42	1874[a]±33	1829[ab]±16	0.003
FCR (feed:gain)	1.69[a]±0.01	1.65[ab]±0.03	1.68[a]±0.03	1.66[ab]±0.02	1.61[b]±0.02	0.004

Means with similar superscript in the same row differ significantly; FCR-feed conversion ratio. T_0-No supplementation in drinking water; T_1- Neem leaves extract supplementation (10 ppm) in drinking water; T_2-Garlic leaves extract supplementation (10 ppm) in drinking water; T_3- Plantain extract supplementation (10 ppm) in drinking water; T_4-Quinosol 10% supplementation (10 ppm) in drinking water.

Plasma metabolites

Herbal extract supplementation with drinking water did not show any positive impact on total cholesterol, high density lipoprotein and low-density lipoprotein contents in experimental birds. Triglyceride and very low-density lipoprotein contents were lower ($p<0.05$) in all the herbal treated groups than control but elevated at AGP group.

Several studies (Aouadi *et al*., 2000; Yeh and Liu, 2001) reported cholesterol lowering effect with the addition of garlic leaves in broiler, which is contradictory with our findings. Moreover, Konjufca *et al*. (1997) noted that garlic leaves powder had significant effect on reduction of plasma triacylglycerols (TGs) in broiler. Likewise, Chowdhury *et al*. (2002) found lower TGs content in layer eggs supplemented with 2 % garlic powder in basal diet and Choi *et al*. (2010) also found lower serum TGs in broiler fed dried garlic leaves. Issa and Omar (2012) investigated that garlic leaves powder decreased triglyceride, LDL and increased HDL levels compared to control in broiler that justify our findings. Main reason for reducing TGs content in our experiment might be due to garlic supplementation decreased 3-hydroxy-3-methylglutaryl reductase activity and cholesterol 7 alpha-hydroxylase activity (Konjufca *et al*., 1997) and ultimately it reduced the TGs content in garlic leaves supplemented group.

Carcass characteristics

Higher abdominal fat content ($p<0.001$) was obtained in the AGP group. Plantain and garlic leaves extracts exhibited reduction of abdominal fat compared to control whereas no change ($p>0.05$) was found in birds supplemented with neem leaves extract. Highest breast meat was found in control group over all the treated groups, but among the treated groups, the proportion was higher in herbal extract groups compared to AGP treated group. Drumstick and thigh muscle percentage were comparatively higher in plantain group, whereas liver content was highest in AGP group. Dressing percent in all the treated groups was higher except for garlic, which was similar to control. No significant variation was depicted in other parameters.

Neem leaves (Grela and Klebaniuk, 2007), garlic leaves (Thampi and Jeyadoss, 2015) and plantain (Al- Mamun *et al*., 2008) are not only the sources of bioactive components but also good sources of proteins and minerals (Al-Mamun *et al*., 2007), which cause higher protein retention and availability leading to better performance and yielded carcass in broiler. Acteoside that are present in plantain (Al-Mamun *et al*., 2007) stimulates leptin expression and cholesterol catabolism, suppressing fatty acid synthesis, and accelerating fatty acid β-oxidation(Yang et *al.,* 2017) and this is one of the reasons for getting lower fat content in plantain supplemented group. Chacrabarti *et al*. (2013) observed positive effects of *Plantago major* L. on carcass characteristics of broiler. Garlic leaves contain a higher concentration of sulfur containing bioactive component like alliin, allicin, S-allylcysteine, diallydisulphide (Onu, 2010) that also behave like acteoside and stimulates leptin expression and cholesterol catabolism.

Table 3: Plasma metabolites of broilers in different dietary treatments

Parameters	T_0	T_1	T_2	T_3	T_4	p-value
TGs (mg/dl)	61.27[ab]±1.95	55.11[ab]±1.63	54.43[b]±1.85	58.54[ab]±3.17	65.57[a]±3.89	0.037
Cholesterol (mg/dl)	86.45±1.28	82.60±1.32	81.33±1.51	80.98±3.30	88.73±2.97	0.063
HDL- C (mg/dl)	53.34±2.90	51.56±2.43	50.79±3.44	50.30±2.88	56.48±2.64	0.324
LDL-C (mg/dl)	20.85±2.01	20.01±3.41	19.65±2.30	18.99±2.73	19.13±2.0	0.942
VLDL-C (mg/dl)	12.25[ab]±0.39	11.02[ab]±0.32	10.88[b]±0.37	11.69[ab]±0.63	13.11[a]±0.77	0.037

Means with similar superscript in the same row differ significantly; TGs, triglycerides; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low-density lipoprotein. T_0- No supplementation in drinking water; T_1- Neem leaves extract supplementation (10 ppm) in drinking water; T_2- Garlic leaves extract supplementation (10 ppm) in drinking water; T_3- Plantain extract supplementation (10 ppm) in drinking water; T_4- Quinosol 10% supplementation (10 ppm) in drinking water.

Table 4: Carcass characteristics of broiler in different dietary treatments

Parameters	T_0	T_1	T_2	T_3	T_4	p-value
Head (%)	3.08	2.83	3.23	3.18	3.13	0.200
Neck (%)	2.79	2.80	2.75	3.13	2.89	0.085
Breast (%)	18.58[a]	18.38[b]	16.51[d]	17.07[c]	16.5[d]	<0.001
Drumstick (%)	4.23[b]	4.29[b]	4.26[b]	4.53[a]	4.09[c]	<0.001
Thigh (%)	7.81[c]	8.27[a]	7.91[b]	8.33[a]	8.29[a]	<0.001
Shank (%)	4.56	4.74	4.79	4.60	4.36	0.112
Heart (%)	0.61	0.64	0.63	0.74	0.65	0.217
Liver (%)	3.39[c]	3.22[d]	3.63[b]	3.67[b]	3.92[a]	<0.001
Kidney (%)	0.23	0.22	0.17	0.18	0.20	0.133
Gizzard (%)	3.13	2.89	2.74	2.87	2.94	0.092
Viscera (%)	7.38	7.52	8.09	7.35	7.69	0.125
Abdominal fat (%)	1.33[b]	1.35[b]	1.25[b]	1.15[c]	2.01[a]	<0.001
Blood (%)	3.31	3.33	4.13	3.19	3.51	0.111
Skin (%)	5.43	7.21	8.18	7.21	7.68	0.075
Feather (%)	8.26	7.01	6.65	7.27	6.93	0.225
DP (%)	60.73[c]	61.56[b]	60.83[c]	61.50[b]	63.52[a]	<0.001

Mean with similar superscript in the same row differ significantly; DP, Dressing percentage. T_0- No supplementation in drinking water; T_1- Neem leaves extract supplementation (10 ppm) in drinking water; T_2- Garlic leaves extract supplementation (10 ppm) in drinking water; T_3- Plantain extract supplementation (10 ppm) in drinking water; T_4- Quinosol 10% supplementation (10 ppm) in drinking water

Table 5: Proximate composition of broiler meat in different dietary treatments

Parameters	T_0	T_1	T_2	T_3	T_4	P-value
Moisture (%)	74.36±0.86	72.68±1.25	73.70±1.11	74.04±2.01	73.97±0.33	0.545
CP (%)	22.38±0.77	22.66±1.15	22.90±0.35	22.47±0.58	22.35±1.01	0.912
CF (%)	0.69±0.04	0.61±0.04	0.74±0.07	0.65±0.21	0.67±0.14	0.765
EE (%)	1.87[a]±0.11	1.42[b]±0.12	1.39[b]±0.14	1.36[b]±0.08	2.08[a]±0.79	<0.001
NFE (%)	73.84±0.84	74.10±1.31	73.80±0.32	74.41±0.65	73.76±0.86	0.867
Ash (%)	1.2±0.09	1.21±0.036	1.16±0.07	1.09±0.05	1.12±0.08	0.374

Mean with similar superscript in the same row differ significantly; CP, crude protein; CF, crude fibre; EE, ether extract; NFE, nitrogen free extract. T_0- No supplementation in drinking water; T_1- Neem leaves extract supplementation (10 ppm) in drinking water; T_2- Garlic leaves extract supplementation (10 ppm) in drinking water; T_3- Plantain extract supplementation (10 ppm) in drinking water; T_4- Quinosol 10% supplementation (10 ppm) in drinking water

Meat composition

Proximate components of meat from different dietary treatments did not show any statistical variation except ether extract (EE). Herbal extract supplementation exhibited the positive impact on lean meat production (Table 5). Higher EE was found in both AGP and control groups. However, there was no variation (p<0.05) in meat EE content among the herbal treated groups. Herbal extract like neem leaves (Choudhary *et al*., 2011), garlic leaves (Thampi and Jeyadoss, 2015), and plantain (Al-Mamun *et al*., 2008a) acts as a strong superoxide anion scavenger and natural antioxidant. Antioxidant prevents auto oxidation in the body for which fat is reduced. Chacrabati *et al*. (2013) found

similar result with plantain herbs as a replacement of commercial antioxidant in broiler feed. Dey *et al.* (2012) found lower serum cholesterol in neem leaves fed laying pullet which also justify our findings. On the contrary, several investigations suggested no significant effect on major carcass components and meat characteristics (Javandel *et al.,* 2008; Onibi *et al.,* 2009; Raeesi *et al.,* 2010) which contradicted our findings. On the other hand, Rahimi *et al*. (2011) found positive result especially lower fat content in broiler meat with different herbal extracts like neem, nishyinda and papaya compared to antibiotics virginiamycin.
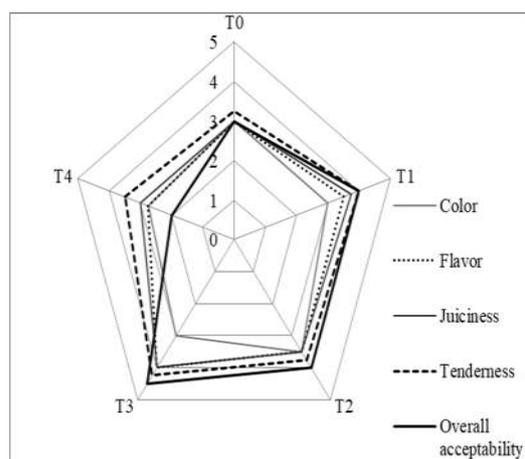
Figure 1: Sensory evaluation of broiler meat in different treatments group

Where, T_0- No supplementation in drinking water, T_1- Neem leaves extract supplementation (10 ppm) in drinking water, T_2- Garlic leaves extract supplementation (10 ppm) in drinking water, T_3- Plantain extract supplementation (10 ppm) in drinking water, T_4- Quinosol 10% supplementation (10 ppm) in drinking water

Sensory parameters

The quality attributes in terms of meat sensory parameters were better in herbal supplemented group (Figure 1) over control and AGP group. This indicated that the supply of herbal extracts improved the sensory qualities of meat. Plantain treated groups exhibited best sensory qualities. Sensory properties of meat are very important in judging the quality of meat. Once the meat is bought, cooked, and served, the aroma, tenderness, juiciness, and flavor must meet the expectations (Aberle *et al*., 2001). Feed for animal have an important effect on this

parameter. When animal is slaughtered oxidation started and antioxidant can stop this oxidation. Oxidation also deteriorates the color and flavor of meat, but if antioxidant rich feed is supplied sufficiently, it will helpful for preserving the quality of meat. All herbal extracts used in this experiment contained higher bioactive components that served as natural antioxidant in biological system and prevented oxidative rancidity of meat. And for this reason, the meat of herbal supplemented group appeared better overall acceptability than AGP and control group.

Conclusion

It may be concluded that the herbal supplements tested in this feeding trial are able to enhance growth and lean meat production in broilers avoiding dependence on antibiotic growth promoter.

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Conflict of interest

The authors would like to declare that there is no conflict of interest

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