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Research Article

Effect of Pharmacologically Active Medicinal Byproduct Combination as Feed Additives on Performance, Fecal Microbiology, Hematological Parameters and Economic Efficacy in Broiler Chicken

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ABSTRACT

An experiment was conducted and designed with pharmacologically active medicinal byproduct combinations (*Camelia sinensis*, *Aloe vera* and *Phylanthus emblica*) as feed additives to check the impact on growth performance, fecal pH and microbiology, hematological parameters and economic efficacy in broiler chicken. A total of 240 chicks were randomly allocated to four treatments having 6 replications of 10 birds per replicated pen following completely randomized design and reared for the period of 5 weeks. Dietary treatments were: 1) MPC0=Control (basal diet without medicinal byproduct combinations); 2) MPC1=basal diet+0.2% medicinal byproduct combination; 3) MPC2=basal diet+0.4% medicinal byproduct combinations; 4) MPC3=basal diet+0.6% medicinal byproduct combination. Result of present study revealed that, medicinal byproduct combinations added groups MPC1, MPC2 and MPC3 exhibited better broiler growth performance as compared to MPC0 ($P<0.05$). Lower fecal pH was depicted in the MPC1, MPC2 and MPC3 inoculated birds in comparison to the MPC0 ($P<0.05$). Suppression of pathogenic *E. coli* was observed in MPC2 and MPC3 and *Salmonella* sp. was observed lower in MPC1, MPC2 and MPC3 supplemented birds relative to that of MPC0 ($P<0.05$). There was no profound negative impact on hematological parameters except lower RBC count was found in MPC2 as compared to MPC0 ($P<0.05$). Economic efficacy was better in case of broilers treated with MPC1, MPC2 and MPC3 compare to MPC0 ($P<0.05$). To sum up, medicinal byproduct combinations with *Camelia sinensis*, *Aloe vera* and *Phylanthus emblica* could be potential feed additives in case of broilers, where MPC2 could be preferred for better efficacy.

Keywords: Broiler chicken; Medicinal byproducts; Growth performance; Fecal microbial loads; Hematological parameters; Economic efficacy

INTRODUCTION

Global health concern about the risk of antibiotics and synthetic growth promoters in livestock and poultry feed triggers the modern conscious consumers to utilize the alternative sources like medicinal plants or probiotics, prebiotics, syntbiotics and other natural feed additives. Medicinal plants are generally considered as a group of plants possessed few special properties that proof them as articles of therapeutic and drugs agents, and are utilized for medicinal purposes. Across the world 12.5 percent plants are reported to have medicinal value among the documented 422000 plant species [1]. Medicinal plants and their extracts are long been used as folk medicine for human health, has a rich history and traditional cultures that have exhibited the primary health care of the local communities based on medicinal functions and related knowledge [2]. Such types of plants naturally synthesize some secondary metabolites like alkaloids, sterols, terpenes, terpenoids, flavonoid, sresins, tennis, lactones, volatile oil and other bioactive compounds. Rising demand of consumers' towards herbal health products and extracts of medicinal plants, has led to an elevated

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production in medicinal plant byproducts all over the world including the developing countries.

According to FAO in 2007 an estimated 121505 tonnes of medicinal plants and aromatic products extracted globally out of which 90181 tones are from Asia. Utilization of medicinal plants and production of medicinal plant byproducts are increasing, where it is reported that, annually this figure is expanding by 15 to 20 percent. Chang [3] reported that, global production of green tea was approximately 1.7 million tonnes in 2013, forecasted to be double in volume by 2023. In Bangladesh, production of tea (*Camelia sinensis*) in 2016 was 58050 metric tons where tea byproducts are being producing every year (Statistical Bulletin- 2017, Bangladesh Tea Board). There are different types of medicinal plant byproducts are also producing in Bangladesh due to elevated utilization of medicinal plants on the aspects of human health. After water, tea is the most widely consumed drink in the world as well as in Bangladesh, as an aromatic beverage commonly tea is prepared by pouring hot or boiling water over curved leaves of Camelia tree, where large amount of byproducts are generating, unutilizing and creating the environmental pollution.

Generally, medicinal plants contain various substances such as antimicrobials, stimulants of immune system, antiviral, and antioxidative properties which can be beneficial for animal health. Similarly, medicinal byproducts may have positive impact in animal health and physiology by providing phenolic compounds, decrease in stress and an improvement in their ultimate productivity and animal product quality. Green tea (*Camelia sinensis*) under the family of *Theaceae* contains a number of bioactive ingredients, including polyphenols such as vitamins, minerals, epigallocatechin gallate and caffeine, [4,5] possess free radical scavenging properties and antioxidative functions [6], source of polyphenols and antioxidants due to abundance of catechins among the phenolic compounds, and possess cancer preventing agents [7]. Fujimura [5] reported that, the major catechin, epigallocatechin gallate in green tea believed to be the primary source of green tea's beneficial effects.

Aloe vera (L.) is another popular local herbal drink in Bangaldesh as vending, where *Aloe vera* waste is produced and creates environmental hazards as well. *Aloe vera* is a succulent plant species of the genus *Aloe* and family *Asphodelaceae*, is the most commercialized *Aloe* species and processing of the leaf pulp has become a large worldwide industry. In the food industry, it has been used as a source of functional foods and as an ingredient in other food products, for the production of gel-containing health drinks and beverages. *Aloe vera* composed of 75 potentially active constituents such as enzymes, vitamins, minerals, lignin, sugars, saponins, salicylic acids and amino acids [8]. It is also found in many consumer products, skin lotion, cosmetics, or ointments for minor burns and sunburns. The raw pulp of *Aloe vera* contains approximately 98.5% water, while the mucilage or gel consists of about 99.5% water [9]. The contemporary part is 0.5-1% solid material contains a range of components including fat-soluble and water-soluble vitamins, minerals, polysaccharides, enzymes, phenolic compounds and organic acids [10]. It has been hypothesized that diverse pharmacological and therapeutic activities can be shown by having such types of heterogenous composition of the *Aloe vera* pulp [11]. It is proclaimed that the *Aloe vera* polysaccharides possesses therapeutic properties such as anti-inflammatory effects, wound healing, immunostimulation, promotion of radiation damage repair, anti-bacterial, anti-viral, anti-diabetic, anti-fungal and anti-neoplastic activities, stimulation of hematopoiesis and anti-oxidant effects [11,12].

Gooseberry (*Phyllanthus emblica Linn., Emblica officinalis Gaertn.*) *Phyllanthus emblica* is available and commonly used herbal medicine in Bangladesh belongs to the *Euphorbiaceae* family, very rich in vitamin C and contains different types of minerals and vitamins such as calcium, iron, phosphorus, vitamin B complex and carotene. The health benefits of *Phyllanthus emblica* can be partially attributed to its high vitamin C content. Variya [13] stated that, the fruit of *Phyllanthus emblica* contains several bioactive phytochemicals, of which majority are of polyphenols (ellagic acid, chebulinic acid, gallic acid, chebulagic acid, apeigenin, quercetin, corilagin, leutolin, etc.). Sugar-substituted phenolics such as phenolic glycosides, flavone glycosides and flavonol glycosides as well as tannins, such as emblicanin A, emblicanin B, phyllaemblicin B, and punigluconoin, are reported in fruit's pulp [13,14]. Because of its chemical constituents, it helps to enhance the absorption of food, balances the acids into the stomach, fortifies the liver, nourishes the brain, improves the mental functioning, and support for the normal functioning of heart. It also hardens the lungs, enhances fertility, regulates elimination of free radicals, helps the urinary system, improves the quality of skin, and promotes healthier hair [15]. This fruit acts as a body coolant, flushes out toxins, improves muscle tone, increases vitality, aids in vision care, and acts as an antioxidant, provides remedies for a lot of diseases and widely utilized in Ayurvedic treatments [16].

Different types of synthetic chemicals are utilizing for the promotion of growth of animal and their products, while they are creating health hazards for the human being [17,18]. To develop potential feed additives, with a view to avoid utilization of synthetic growth promoters, a considerable effort is being paid on combinations of medicinal plants or their byproducts [19,20]. Most agro-industrial byproducts are considered waste products that resulted environmental pollution, even though they contain utilitarian primary and secondary plant metabolites. In addition, there are wide varieties of medicinal plant around the world. Studies have reported an increased body gain and improved meat quality when the effects of using medicinal plant byproducts on swine and poultry diets were evaluated [21,22]. A significant improvement in the growth performance, immunity and suppression of pathogenic caecal *E. coli* was reported with dietary supplementation of *Ginkgo biloba* and *Camelia sinensis* after fermentation as potential feed additives in broiler nutrition in previous study [18].

Bostami et al. [19] concluded that pomegranate byproduct supplementation can potentially improve growth performance, reduce fecal pH and gas emission from excreta, and economically effective in case of broiler. *Phyllanthus emblica* can improve the performance and reduce fecal microbial population and diarrhea prevalence in growing sheep [23]. Previous studies suggested that supplementing diets with phytochemicals exhibiting antioxidant properties may ensure better productivity, immune response and animal products, consequently enhance meat quality traits of chicken, pig and cattle [24]. Utilization of medicinal byproducts can be potential feed supplement approach with better economic efficacy for fattening Hanwoo cattle [25]. Pomegranate byproduct supplementation with the basal diet can be economically effective in case of broiler [19].

It is generally considered that, proper utilization of plant byproducts can minimize the feed cost, where promotion of health and animal product quality enhancement could be the bonus for animal production. Using medicinal plant byproducts as feed additives to livestock and poultry diets could be an economically feasible alternative and eco-friendly option as low cost feed additives or substitutes for basal feed materials [26,27], and may aid in lessen the improper discarding of medicinal plant byproducts as environmental pollutants. Medicinal byproducts could be utilized in the chicken diet as well, since they are composed of primary and secondary bioactive compounds; hence, it was expected that a combinations of medicinal byproducts would have synergistic effects on birds in the current study. However, to the best of our knowledge no study have been investigated the effects of a combination of byproducts of *Camelia sinensis*, *Aloe vera* and *Phylanthus emblica* on broiler chicken nutrition to date. Therefore, the present study was conducted to investigate the effects of combination of byproducts of *Camelia sinensis, Aloe vera* and *Phylanthus emblica* with different ratio on the growth performance, hematological parameters, fecal microbiology and economic efficacy in broiler chicken.

METHODS

Preparation of medicinal byproduct combination with *Camelia sinensis, Aloe vera and Phylanthus emblica*

Camelia sinensis, Aloe vera and Phylanthus emblica was collected from local markets of Gazipur, Peoples Republic of Bangladesh. *Camelia sinensis* byproduct was collected from tea shops of BSMRAU area, cleaned and oven dried for further use. The byproduct of *Aloe vera* was collected from joydebpur vending juice shops, chopped, grinded and refrigerated for further utilization as feed additives. *Phylanthus emblica* fruits was collected from salna bazar, washed with water, sun dried, and oven dried, grinded and poured in bags for further use. *Camelia sinensis, Aloe vera and Phylanthus emblica* were dried in a forced air oven (Doori TEC, Doori TEC, FA, Co., Ltd.) at 80°C for 3 d and subsequently ground into powder that could pass through a 0.15 mm sieve using a milling machine.

All dried materials was mixed and grinded (grinder followed by hand mixing) in the ratio of 50:30:20 to make the medicinal byproduct combinations with a view to minimize the cost feed additives and maximize the utilization of waste material with optimizing antioxidative potentials for broiler diet. Dried and grinded samples were then tightly packed in glass container and kept at room temperature until use. The combination of byproducts of *Camelia sinensis, Aloe vera and Phylanthus emblica* was termed as “Medicinal Plant Byproduct Combinations (MPC)”. *Camelia sinensis, Aloe vera and Phylanthus emblica* samples were analyzed in triplicate for crude protein (CP), ether extract (EE), moisture and ash as described by the Association of Official Analytical Chemists (AOAC, 2000). The pH was measured using a digital pH meter (Hanna pH meter, Romania), where sample was ranged between 3 to 4.

Experimental design, dietary treatments and husbandry practice

Effect of medicinal plant combinations on performance, fecal microbiology, hematological parameters and economic efficacy was measured in case of broiler chicken. Cobb-500 one day-old healthy chicks were collected from local hatchery. A total of 240 chicks was randomly allocated to 4 treatment groups having 6 replications of 10 birds per replicated pen. All birds were randomly allocated to different dietary treatment groups following completely randomized design and reared for the period of 5 weeks. Where mash and crumbles feed was provided to the birds dividing starter (0 to 3 weeks) and finisher (4 to 5 weeks) group to meet the nutrient requirement of broiler chicken following NRC (1994) and Cobb-500 commercial broiler management manual (NRC, Nutrient Requirements of Poultry. 9th revised ed. National Research Council, National Academy of Science, Washington, DC. 1994). Feed ingredients and chemical compositions of the basal diets was shown in Table 1. Medicinal byproduct combinations were added according to the treatments. Dietary treatments were: 1) MPC0=Control (basal diet without medicinal byproduct combinations); 2) MPC1=basal diet+0.2% medicinal byproduct combination; 3) MPC2=basal diet+0.4% medicinal byproduct combinations; 4) MPC3=basal diet+0.6% medicinal byproduct combination. Animal care and management was followed according to the suggestion of the Animal care and management committee of Faculty of Veterinary Medicine and Animal Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh, following the guides, policies, rules and regulations of Department of Livestock Services, Peoples Republic of Bangladesh.

All birds were reared in a floor with hygienic saw dust, which were routinely stirred and dried to maintain the healthy condition. The house was well ventilated, aerated, having proper lighting. Feeder and waterer was distributed according to the bird’s requirements. Ad libitum feed and fresh clean drinking was ensured four times daily. Biosecurity measures was followed properly to safe the birds from any types of contamination or disease outbreak. Temperature was maintained by providing bulb as well as room heater according to the Cobb-500 broiler user guide and manual. During experimental period, body weight, body weight gain, feed intake and feed conversion ratio was measured and calculated weekly basis. Fecal sample and blood sample was collected at the end of the experimental period for different parameters analysis. For economic analysis, cost per unit of gain was calculated.

Determination of phenolic content

A 5g sample was weighed properly and were extracted continuously for 4 hours in a Soxhlet apparatus. Where extraction was done by using ethanol, ethyl ether or deionized water (100 mL) at 76°C, 40°C and 97°C respectively. Following genuine extraction period, the volume was completed to 100 mL with the appropriate solvent. Then the samples were kept in amber vessels under nitrogen atmosphere at -18°C until the analyses were executed. All procedures were carried out using three independent replicates. The determination of phenolic content was done following the methodology applied by Bastos et al. [28]. In brief, Folin-Ciocalteau reagent was used for the determination of total phenolic content [29]. Results were expressed as chlorogenic acid equivalents. Phenolic compounds were identified by negative ion mode electrospray mass spectrometry (ESI-MS) fingerprinting. The extracts were analyzed by direct infusion directly into the source by means of a syringe pump (Harvard Apparatus). Where the flow rate was maintained of 10 mL/ min. ESI–MS fingerprints of the extracts and tandem mass spectra (ESI-MS/MS) were acquired in the negative ion mode using a hybrid high-resolution and high-accuracy (5 ppm) Micromass-Waters Q-TOF mass spectrometer (Manchester, UK). Capillary voltage was set to -3000 V and cone voltage was set to -40 V. Where the desolvation temperature of 100°C was maintained. A collision energy was optimized for each component where it was varied from 15 to 50 V for tandem mass spectra (ESI-MS/MS) of mass selected compounds. The extracts were diluted in a solution containing 70% (v/v) chromatographic grade methanol (Tedia, Fairfield, OH, USA), 30% (v/v) deionized water and 0.5% of ammonium hydroxide (Merck, Darmstadt, Germany). The analysis of the extracts were done by direct insertion negative ion mode ESI-MS fingerprinting. The method furnishes a sensitive and selective methodology for the singling out of polar organic compounds with acidic sites, such as the phenolic compounds found in MPC. Compounds of interest were then mass selected and their ESI-MS/MS contrasted to those found in references, for the recognition of these compounds. The phenolic content (mg/mL) of MPC were observed 3 to 5 in water solvent, 7 to 10 in ethanol solvent and 0.03 to 0.06 in ether solvent.

Measurement of growth performance

Continuous lighting was provided for the entire experimental period, and there was followed user's manual vaccination or medication program. Chicks were inspected daily and dead birds were removed following recoding of the mortality (pen, date and body weight). Body weight (BW) was recorded weekly by replicate, feed intake (FI) was calculated by deducting feed residue from total feed supplied in each replicated pen, average daily gain (ADG), the average daily feed intake (ADFI), and FCR (feed conversion ratio) per replicated pen were then calculated by period and for the total experimental period.

Collection and analyses of fecal samples

Fecal microbial loads was measured and analyzed following the procedure explained by Kim et al. [18] and Bostami et al. [30]. In brief, during 5th week of experimental period, fecal samples were collected from each replicated pen for fecal pH and microbial load analysis. Fecal samples were collected from each replicated pen and mixed properly for homogeneity and removed the foreign particles or fathers. The collected fecal samples was serially diluted in sterile saline in the 1:10 dilution and then cultured on agar media (duplicate for each). Total bacteria, *Lactobacillus sp., Salmonella sp.* and *E. coli of* fecal sample were measured by using specific agar media. The culture media for total bacterial, *Lactobacillus spp. E. coli, Salmonella spp.,* were Nutrient agar, Lactobacilli MRS (Mann, Rogosa and Sharpe) Agar, MacConkey Sorbitol Agar; *Salmonella* Shigella Agar; respectively (DIFCO-TM, USA). Agar plates were incubated in anaerobic condition at 37°C for 24 h (*E. coli and Salmonella*) and 48 h (Total bacteria and *Lactobacillus spp.*) were done followed by the smearing of supernatant of 100 μl onto the agar plate. Enumeration of microbial colonies in the duplicate incubated agar plates were done and microbial loads was counted and were expressed as log10 CFU/ml.

Collection and analyses of blood samples

Hematological parameters: At 36 days of age, two birds were chosen randomly from each replicate for blood sample collection. The 3 mL of blood was collected from wing vein from two birds of each replicate into anticoagulant EDTA treated tubes for determination of total Red Blood Cell (RBC), total White Blood Cell (WBC), hemoglobin (HB), hematocrit (HCT) and other determinants. The following hematological parameters were assessed by using Automatic Fully Digital Hematology Analyzer, BC- 3000 Plus, Shenzhen Mindray, Bio-Medical Electronics Co., LTD.

Economic analysis

After rearing of the birds for 5 weeks of age, segmented into two phases, starter and finisher, feed cost per unit of gain was calculated for economic efficacy of utilization of medicinal plant combinations in case of broilers.

Statistical analysis

All data was subjected to ANOVA using the General Linear Models (GLM) function of the Statistical Analysis System (SAS, 2003, Version 9.1, SAS Institute, Cary, NC, USA). Each cage was considered as the experimental unit for growth performance parameters (BW, ADG, ADFI and FCR) and economic analysis; whereas an individual bird was served as the experimental unit for blood and fecal microbial analysis. A probability level of $P<0.05$ was considered as statistically significant and a level of $P<0.10$ was considered as statistical tendency.

RESULTS

Growth performance of broiler chicken

Table 2 shows the effect of medicinal byproduct combinations on growth performance of broiler chicken. It was observed that, final body weight was higher in MPC2 and MPC3 inoculated birds as compared to MPC0 ($P<0.05$). Body weight gain was higher in MPC2 and MPC3 inoculated birds as compared to MPC0 ($P<0.05$) during 4 to 5 weeks and 0 to 5 weeks of experimental period; where MPC1 did not differ with MPC0 during 4 to 5 weeks and 0 to 5 weeks of experimental period ($P>0.05$). Feed conversion ratio (FCR) was higher in the MPC3 added birds in comparison to MPC0 ($P<0.05$) during 0 to 3 weeks, however, during 4 to 5 weeks and 0 to 5 weeks lower FCR was exhibited in the MPC1, MPC2 and MPC3 inoculated birds relative to that of MPC0 ($P<0.05$). There was observed no difference within the MPC supplemented birds during 4 to 5 weeks and 0 to 5 weeks of experimental period on the aspect of FCR.

Fecal microbial loads and pH of broiler chicken

Effect of medicinal plant byproduct combinations on fecal microbial loads (log10 CFU/g) and pH of broilers was shown in Table 3. Where result revealed that, *E. coli* was suppressed in MPC2 and MPC3 treated birds as compared to MPC0 ($P<0.05$), where MPC1 did differ with MPC2 and MPC3 ($P<0.05$). *Salmonella* sp. was diminished in MPC1, MPC2 and MPC3 inoculated birds relative to that of MPC0 ($P<0.05$), where MPC1, MPC2 and MPC3 inoculated birds did not differ within the groups ($P>0.05$). Lower fecal pH was found in the MPC1, MPC2 and MPC3 treated birds as compared to MPC0 ($P<0.05$), where within the MPC groups there was no significant difference ($P>0.05$).

Table 1: Feed ingredients and chemical compositions of the basal diets.

Items	Starter diet (0 to 3 weeks)	Finisher diet (4 to 5 weeks)
Ingredients (g/kg as fed basis)		
Corn grain	575.8	606.4
Soybean meal	268.0	249.0
Corn gluten	50.0	35.0
Soybean oil	22.0	22.0
Animal fats	45.0	50.0
Common salt	2.5	2.5
Dicalcium phosphate	21.4	20.0
Limestone	9.2	8.8
Vitamin-mineral premix 1	3.0	3.0
Choline	0.8	0.7
L-lysine HCl (78%)	2.4	1.6
DL-Methionine	2.0	1.0
	Calculated composition (g/kg DM)	
ME (MJ/kg)	130.3	132.7
Moisture	120.7	130.8
Crude protein	208.9	191.2
Ether extract	46.5	24.3
Crude fiber	44.2	37.1
Crude ash	56.3	56.1
Calcium	10.5	8.1
Available phosphorus	5.5	4.5
Lysine	14.2	11.0
Methionine	4.9	4.5

[1]Vitamin-mineral mixture provided the following nutrients per kilogram of diet: vitamin A, 15,000 IU; vitamin D3, 1,500 IU; vitamin E, 20.0 mg; vitamin K3, 0.70 mg; vitamin B12, 0.02 mg; niacin, 22.5 mg; thiamine, 5.0 mg; folic acid, 0.70 mg; pyridoxine, 1.3 mg; riboflavin, 5 mg; pantothenic acid, 25 mg; choline chloride, 175 mg; Mn, 60 mg; Zn, 45 mg; I, 1.25 mg; Se, 0.4 mg; Cu, 10.0 mg; Fe, 72 mg; Co, 2.5 mg.

Table 2: Effect of medicinal byproduct combinations on growth performance of broilers.

Parameters		MPC0	MPC1	MPC2	MPC3	SEM	P-value
IBW (kg/bird)		0.045	0.046	0.045	0.046	0.001	0.387
FBW (kg/bird)		1.937^{c}	2.020^{bc}	2.276^{a}	2.209^{ab}	0.062	0.020
BWG (kg/bird)	0 to 3 week	0.978	0.962	0.962	0.864	0.045	0.367
	4 to 5 week	0.914^{b}	1.011^{b}	1.270^{a}	1.299^{a}	0.044	<0.0001
	0 to 5 week	1.892^{c}	1.974^{bc}	2.232^{a}	2.163^{ab}	0.063	0.019
Feed intake (kg/bird)	0 to 3 week	1.343	1.320	1.348	1.293	0.066	0.935
	4 to 5 week	1.553	1.472	1.705	1.763	0.075	0.195
	0 to 5 week	2.895	2.792	3.054	3.056	0.076	0.112
FCR	0 to 3 week	1.372^{b}	1.372^{b}	1.407^{b}	1.494^{a}	0.011	<0.0001
	4 to 5 week	1.721^{a}	1.476^{b}	1.325^{b}	1.357^{b}	0.068	0.003
	0 to 5 week	1.541^{a}	1.423^{b}	1.371^{b}	1.413^{b}	0.035	0.037

[abc]Means with different superscripts within the same row are significantly different (P <0.05).
IBW: Initial body weight; FBW: Final body weight; BWG: Body weight gain; FCR: Feed conversion ratio. Dietary treatments: 1) MPC0=Control (basal diet without medicinal byproduct combinations); 2) MPC1 = basal diet+0.2% medicinal byproduct combination; 3) MPC2=basal diet+0.4% medicinal byproduct combinations; 4) MPC3 = basal diet +0.6% medicinal byproduct combination.

Blood hematological parameters of broiler chicken

Table 4 shows the effect of medicinal plant byproduct combinations on hematological parameters of broiler chicken. It was depicted that, there was no large impact on hematological parameters due to addition of MPC with the diet of broiler chicken except a lower RBC in MPC2 relative to MPC0 and lower MCHC in MPC3 added group as compared to MPC1 (P<0.05).

Economic efficacy of broiler chicken

Effect of medicinal plant combinations on economic efficacy of broilers was shown in Table 5. Cost per unit of gain was higher in case of MPC3 added group compare to that of MPC0 group (P<0.05) during 0 to 3 weeks. Where, Cost per unit of gain was lower in

Table 3: Effect of medicinal byproduct combinations on fecal microbiology (log_{10}cfu/g) and pH of broilers

Parameters	MPC0	MPC1	MPC2	MPC3	SEM	P-value
Total bacteria	8.762	9.112	8.748	8.808	0.113	0.148
Lactobacillus sp.	7.029	6.750	6.784	6.943	0.070	0.119
E. coli	2.903^{a}	2.788^{a}	2.459^{b}	2.557^{b}	0.072	0.001
Salmonella sp.	3.561^{a}	3.348b	3.360^{b}	3.336^{b}	0.047	0.009
Fecal pH	7.075^{a}	6.420^{b}	5.450^{c}	5.897^{d}	0.049	<0.0001

^{abc}Means with different superscripts within the same row are significantly different (P<0.05).
Dietary treatments: 1) MPC0=Control (basal diet without medicinal byproduct combinations); 2) MPC1 = Basal diet + 0.2% medicinal byproduct combination; 3) MPC2 = Basal diet + 0.4% medicinal byproduct combinations; 4) MPC3 = Basal diet + 0.6% medicinal byproduct combination.

Table 4: Effect of medicinal byproduct combinations on blood biochemical parameters of broilers.

Parameters	MPC0	MPC1	MPC2	MPC3	SEM	P-value
WBC (×10^3mm^3)	203.467	203.389	203.173	203.751	0.501	0.879
HGB (g/dl)	13.611	14.808	13.411	13.941	0.577	0.376
RBC (×10^6/mm^3)	3.601^{a}	3.214^{ab}	2.857^{b}	3.236^{ab}	0.151	0.038
HCT (%)	46.247	46.330	46.323	45.963	0.261	0.770
MCV (fl)	167.300	165.967	169.867	167.845	2.077	0.666
MCH (pg)	46.782	46.533	46.035	46.206	0.545	0.774
RDW_CV (%)	18.608	16.797	17.827	17.479	1.305	0.803
RDW-SD (fL)	149.048	147.974	166.771	159.808	11.176	0.643
PLT (×10^3 /mm^3)	49.452	52.467	55.784	52.877	4.795	0.837
MPV (fL)	13.041	12.544	13.583	12.687	0.636	0.698
PDW (fL)	16.181	16.678	16.083	14.730	0.572	0.267
PCT (%)	0.437	0.440	0.462	0.421	0.013	0.247

^{abc}Means with different superscripts within the same row are significantly different (P <0.05).
RBC=Red Blood Cell/Erythrocytes Count; HGB=Hemoglobin; HCT=Hematocrit; MCV=Mean Cell Volume; WBC=White Blood Cell Count; LYM=Lymphocyte Count; MONO=Monocytes Count; GRA=Granulocytes Count; PCT=Platelet Percent; MPV=Mean Platelet Volume; RDW=Red Blood Cell Distribution Width. The RDW-CV is a calculation based on both the width of the distribution curve and the mean cell size (%). The RDW-SD is an actual measurement of the width of the red cell distribution curve in femtoliters (fL). MPV: Mean platelet volume (fL (femtoliters)). The average volume of a platelet; newer platelets tend to be larger than older ones. PDW: platelet distribution width (fL (femtoliters)). Mean corpuscular volume (MCV): Average size of red blood cells (fL). Hematocrit (HCT): The percentage of red blood cells (%). Mean corpuscular volume (MCV): Average size of red blood cells (fL). Mean corpuscular hemoglobin (MCH): The amount of hemoglobin per red blood cell (pg). Mean corpuscular hemoglobin concentration (MCHC): The average concentration of hemoglobin in a given volume of red blood cells (g/dL). Platelet count (PLT). Mean platelet volume (MPV). PCT is the volume occupied by platelets in the blood as a percentage and calculated according to the formula PCT = platelet count × MPV/10,000 (25-27). Under physiological conditions, the amount of platelets in the blood is maintained in an equilibrium state by regeneration and elimination.
Dietary treatments: 1) MPC0=Control (basal diet without medicinal byproduct combinations); 2) MPC1=Basal diet + 0.2% medicinal byproduct combination; 3) MPC2=Basal diet + 0.4% medicinal byproduct combinations; 4) MPC3=Basal diet + 0.6% medicinal byproduct combination.

Table 5: Effect of medicinal byproduct combinations on economic efficacy of broilers (USD).

Parameters	MPC0	MPC1	MPC2	MPC3	SEM	P-value
0 to 3 wk	0.728^{b}	0.730^{b}	0.750^{b}	0.798^{a}	0.006	<0.0001
4 to 5 wk	0.914^{a}	0.786^{b}	0.706^{b}	0.725^{b}	0.036	0.004
0 to 5 wk	0.818^{a}	0.757^{ab}	0.731^{b}	0.755^{ab}	0.019	0.046

^{abc}Means with different superscripts within the same row are significantly different (P <0.05).
Dietary treatments: 1) MPC0=Control (basal diet without medicinal byproduct combinations); 2) MPC1=Basal diet + 0.2% medicinal byproduct combination; 3) MPC2=Basal diet + 0.4% medicinal by-product combinations; 4) MPC3=Basal diet + 0.6% medicinal byproduct combination.

MPC1, MPC2 and MPC3 in comparison to MPC0 (P<0.05) during 4 to 5 weeks; and during 0 to 5 weeks of experimental period, cost per unit of gain was around 11% lower in MPC2 relative to that of MPC0 (P<0.05). Where, MPC incorporated birds did not differ within the group on the aspect of cost per unit of gain during 4 to 5 weeks and 0 to 5 weeks of experimental period (P>0.05).

DISCUSSION

Effect on growth performance of broiler chicken

The fashion of traditional medicine use is as old as the human race itself, where Adam et al. [31] proclaimed that modern herbal drink is elevating due to health aspects with gaining the functional and pharmacological benefits. It has been estimated that over 60% of the current drugs especially the antihypertensive and anticancer drugs are originated from plant [32]. Medicinal plants have therefore become principal source of research and development of new drugs [33]. Currently, for potential therapeutic effects many plants are being investigated having evidence of anticancer, antitussive, anti-arthritic, analgesic, anti-inflammatory, antitumor, antibacterial, antioxidant, antifungal, antihistaminic, antidiabetic, lipid-lowering, laxative, hepatoprotective and acetylcholinesterase inhibitory [31]. Medicinal plants are copious in different geographical locations of the world including Bangladesh, India, Korea, Japan and China. The use of alternative medicines, particularly the herbal products elevated forcefully during the last decade in the United States as well as in the other countries. According to the estimates of the World Health Organisation (WHO) traditional medicine use is up to 80% of the population in some developing countries. In addition, trade in herbal medicine, use of herbal drinks is gaining acceptance globally and is now a lucrative business generating lots of revenue, consequently creating a wider scope of generation of medicinal plant byproducts.

It was reported that, *Citrus junos* and *Punica granatum* byproducts efficiently can improve weight gain in broilers when they supplemented separately [19]; *Ginkgo biloba* and *Camelia sinensis* after processing have no effect on weight gain of birds when they individually supplemented with diet of animals [34,35]. Mehala & Moorthy [36] reported no significant difference in body weight and body weight gain between treatment groups from first week to end of the experiment after supplementation of *Aloe vera* and *Curcuma longa*. A study was conducted to investigate the effect of feeding lemon balm (*Melissa officinalis*) and *Aloe* (*Aloe vera*) on growth performance of rainbow trout [37]. However, in the current study, the better growth performance (body weight gain and FCR) was exhibited in the MCP treated broilers might be attributed to the presence of plant secondary metabolites synergistic actions that were generated from the byproduct of *Camelia sinensis, Aloe vera* and *Phylanthus emblica*. Where it was depicted that, higher dose was more effective compared to lower dose of MCP inoculation with the basal diet of broiler chicken. The result indicated that, *Aloe vera* might be matched better with *Camelia sinensis* and *Phylanthus emblica* rather than the previous studies due to their phenolic compounds and flavonoids.

The polyphenolic compounds of green tea have been shown to improve body weight gain and feed efficiency in case of livestock and poultry [35]. Sarker et al. [35] suggested that, diets with addition of 0.5% green tea and 1.0% fermented green tea probiotics groups were suitable for broiler growth performance. Kim et al. [18] asserted that average daily gain was higher in *Ginkgo biloba* and *Camelia sinensis*-based fermented products supplemented group; where feed intake was unaffected after dietary supplementation. However, feed conversion ratio was better in fermented *Ginkgo biloba* and *Camelia sinensis* in comparison to control group [18]. Sarker et al. [35] reported significant improvement of weight gain in broilers during the finishing period at 0.5% level relative to 1.0% level of green tea. It was stated increase in body weight when birds were supplemented with polyherbal feed premix containing *Emblica officinalis [38].* Chaudhary et al. [39] proclaimed that, the weight gain of basal diet with 1.0% *C. longa, E. officinalis* and *N. sativa* powder mixture group broilers were significantly higher than basal diet without any supplement group. Basal diet with antibiotic and basal diet with 1.0% *C. longa, E. officinalis* and *N. sativa* powder mixture groups showed significantly lower FCR than negative control group [39]. Where, present study, described that, byproducts of *Camelia sinensis* in combination with *Aloe vera* and *Phyllanthus emblica* potent in escalating the body weight gain and better FCR of broiler chicken.

It was reported that, dietary supplementation of *E. officinalis* at 0.4% and 0.8% levels did not show any adverse effect on feed intake [40]. Patil et al. [41] reported that significant increase in feed intake when birds were supplemented with either *E. officinalis* fruit powder alone or in form of poly-herb; where Gaikwad et al. [42] affirmed that, chicks with 1% *Emblica officinalis* supplementation attained lower feed intake. Feed consumption was lower in herbal supplemented broilers than negative control group [39]. Present findings revealed that, that combinations of *Camelia sinensis*, *Aloe vera* and *Phyllanthus emblica* at 0.2%, 0.4% and 0.6% level with basal diet of broiler exhibited no negative impact on feed intake. The higher body weights, better FCR and no negative impact of feed intake observed in MPC supplemented groups may be attributed to the presence of anabolic and antioxidant effect of minerals, amino acids, ascorbic acid, gallic acid, tannic acids and phenolic compounds generated from *Camelia sinensis*, *Aloe vera* and *Phyllanthus emblica.* The varying result of the present study on the aspect of growth performance of broiler might attributed to the different ratio of 50:30:20 for byproduct of *Camelia sinensis*, *Aloe vera* and fruits of *Phyllanthus emblica* respectively. Present study ensures that, byproduct of *Camelia sinensis*, *Aloe vera* and fruits of *Phyllanthus emblica* can efficiently boost the basal diet of broiler with escalation of the performance indices.

Effect on fecal microbial loads and pH of broiler chicken

The actual mode of action of different medicinal plants or byproducts generated bioactive constituents is hard to determine due to the wider functions into the gastro intestinal tract of ruminants and nonruminants [43]. Previous studies on the effects medicinal plants and their byproducts on performance indices showed varying results in case of livestock and poultry [43]. Where modulation of microbial phenomena inside the gastrointestinal tract is the major factor that actually depicted the positive or negative impact on the performance indices of livestock or poultry. The polyphenolic compounds derived from medicinal products or byproducts can maintain microflora harmonize and manifest antimicrobial effects contrary to pathogenic bacteria [44,45]. Dietary supplementation of fermented *Ginkgo biloba* and fermented *Camelia sinensis* at 0.2% and 0.4% level significantly suppressed caecal pathogenic *E. coli* [18]. Kim et al. [18] suggested that dietary fermented *Ginkgo biloba* and fermented *Camelia sinensis* can be utilized as potential feed additives in broiler nutrition with significant suppression of pathogenic caecal *E. coli.*

Guray et al. [44] concluded that the improved production results in the broilers with added green tea extract are directly connected with physiological mechanisms such as the regulation of the caecal microflora. Green tea has over 200 bioactive compounds and contains over 300 different substances. The chemical composition of tea is multifaceted, consisting of polyphenols (catechins and flavanoids), alkaloids (caffeine, threobromine, theophylline), volatile oils, polysaccharides, amino acids, lipids, vitamin C, minerals and other uncharacterised compounds. The improved FCR in broiler chickens fed diet MPC1, MPC2 and MPC3 could be attributed to the effects of plant derived bioactive compounds, which mediated reduction of pathogenic microbes (*E. coli* and *Salmonella*) and improved utilization of nutrients in the digestive tract. Improvements in FCR have been observed upon treatment with concentrated grape pomace containing resveratrol [46] through modulation of gastrointestinal microflora.

Aloes have been widely used for a broad range of pharmacological activities and in resolving the microbial or parasitic infestation [47]. The protective effects of *Aloe vera*-based diets were assessed in case of broiler chicken following oral infection with *Eimeria maxima*. Fecal oocyst shedding can diminuted significantly in all of the supplemented group with *Aloe vera* as compared to the unsupplemented group. Yim et al. [47] reported that, the *Aloe vera*-supplemented group showed significantly fewer intestinal lesions than that of the unsupplemented group following infection. The findings of the Yim et al. [47] study concluded that *Aloe vera* can be used as an alternative treatment for controlling avian coccidiosis or *Eimeria spp.* infections. Present findings suggested that, *Aloe vera* in combination with *Camelia sinensis* byproducts and *Phyllanthus emblica* is potential for intestinal microbial balance which was reflected in the fecal material of broiler chicken. Phytogenic acid, polyphenolic compounds and flavonoids like tannic acid generated from medicinal plants or their byproducts are able to diminish the faecal coliform bacterial count without influencing the other faecal bacteria [48,49]. Polyphenols, hydrolysable tannins and flavonoids of the medicinal plants can exhibit antimicrobial action which can diminish the fecal microbial count [50]. *In vitro* study of medicinal plant containing higher concentration of hydrolysable tannins, flavonoids and polyphenols efficiently acts against pathogenic *E. coli* and *Salmonella* population [51-53]. The low pH of medicinal plant containing flavonoids (pH 3 to 4) may also create an acidic condition in the gastrointestinal tract of broiler which prevents the growth of pathogenic bacteria.

Previous studies of rats and broilers have shown that dietary plant polyphenols induce a shift in the microbial populations of the intestinal tract [54]. According to Scalbert [55], tannins exert antimicrobial activity by inhibiting extracellular microbial enzymes and removing substrates required for microbial growth, resulting in disruption of the membrane structure and function. In this study, dissipation of the intestinal pH gradient together with the antimicrobial activity of phenolic tannins may have been responsible for the reduction in *E. coli* and *Salmonella sp.*. The antimicrobial activity of medicinal combinations against excreta microbiology was more pronounced when increasing dietary levels. The observation of lower concentrations of *E. coli* and *Salmonella* spp. and lower pH of the feces of broiler chicken in the present findings can be explained as a reflection of intestinal microbiology and pH due to phenolic compounds generated from byproducts of medicinal plants [50].

Effect on hematological parameters of broiler chicken

Hematological and biochemical tests are an important tool that could be used in livestock, poultry or fish health assessment. Several research on hematological studies of nutritional effects, pollutants and infectious diseases indicated that erythrocytes are a major and reliable indicator of various sources of stress. Moreover, generally RBCs convey hemoglobin (Hb) that, in turn, carry oxygen, where the amount of oxygen received by tissue gamble on the RBCs maturity and Hb amount [56]. The higher RBC and WBC counts was proclaimed in the Nile tilapia fed green tea compared to control group, where they suggested that green tea able to boost the hematological function of the fish [56].

Aloe vera and *Curcuma longa* and its combination was added with broiler diet, where there was found difference on hemagglutination inhibition titre level in the treated birds while compared with the control diet [57]. A study was conducted to investigate the effect of feeding lemon balm (*Melissa officinalis*) and *Aloe* (*Aloe vera*) on hematological parameters of rainbow trout [37]. Survival rate of fish was promoted in diets supplemented with herbs, and no significant difference was exhibited in case of RBC and Hb in treated groups [37]. The study evaluated the effect of dietary supplementation of *Camellia sinensis* leaf-extract on non-specific immune responses and disease resistance of *Mugil cephalus* fingerling against *P. damselae*. Fish were fed with 0 (unsupplemented), 50, 100 and 200 mg/kg of green tea extract supplemented diets. Results indicated that green tea extract decreased mortality in *M. cephalus* in a dose-dependent manner after challenge with *P. damselae*. Haematological parameters containing RBC, Hct, Hb and WBC and growth performance (weight gain) showed remarkable changes in comparison with control group [37,58]. However, in the present research, it was reflected that, hematological parameters were not profoundly affected after administering the MPC in the diet of broiler chicken except the alteration of RBC count in the MPC2 compared to MPC0. Actual mode of action of the MPC2 is not clear in this study, nevertheless, the polyphenols and flavonoids, or the specific mineral contents can exhibit impact on the RBC of broiler.

Tannic acid can reduce the hematological indices [48]. Total erythrocyte count, hemoglobin, hematocrit and plasma iron concentration can reduce with higher levels of tannic acid in the diet of pig [48]. Inclusion of tannic acid in the diet of pig reveals negative impact on performance, hematological indices and plasma iron status [48]. *Camelia sinensis* polyphenols stoichiometrically bind ferric iron to form a redox-inactive Fe- polyphenols complex. Catechins from the tea polyphenols are the likely iron-binding compounds accounting for the antioxidant effects of tea polyphenols on RBC [59]. Polyphenols along with other ions can cause decrement of hematological parameters. Spectrophotometric titration revealed that tea polyphenolic compounds could stoichiometrically bind ferric iron to form a redox-inactive Fe-*Camelia sinensis* polyphenolic complex. Quantitative analysis suggests that one or more major catechins from the *Camelia sinensis* polyphenolic compounds preparations are the likely iron-binding compounds accounting for the antioxidant effects of *Camelia sinensis* polyphenolic compounds on RBC [59]. Polyphenols along with other ions can cause decrement of hematological parameters.

Effect of Soursop (*Anona muricata L.*) on blood hematology and serum biochemistry of Sprague-Dawley rats reported that, Soursop (*Anona muricata L.*) with different dose (0,0.5,1.0 and 2.0g/kg bw) did not show negative effect in blood hematology except increment of platelet level [60]. Although medicinal plants or their byproducts should be used with care because elevated supplementation can stimulate undesirable side effects for animals. Previous research findings, disclosed that, hematological parameters can be affected by the phytogenic compounds generated from the medicinal plants in case of animal or fish study. In the current study, hematological parameters was unaffected except the lower RBC was depicted in the MPC2 group compared to MPC0. Result indicated that, there was no negative impact of utilization of MPC in the broiler chicken diet. However, the lower value of RBC is not clear, warrants further detail study to discover the mechanism.

Effect on economic efficacy of broiler chicken

Use of different medicinal plants results in a large amount of byproducts most of which are currently discarded, leading to environmental pollution; however, there is the potential for these by-products to be used as feedstuff for livestock or poultry [61]. Consequently, utilization of byproducts in the broiler chicken diet can ensure better economic efficacy along with the plant secondary metabolites. Pomegranate byproducts can ensure better growth performance and economic efficacy in case of broiler [19]. The green tea leaves, by-products, flowers, extracts and polyphenols can be afford as an ingredient or as a supplement to broiler diet for diminishing the mortality in diseased condition of birds [62] to poultry diets can be subsidized for enhancing performance indices and inducing the plasma parameters [63]; all of which in turn able to influence efficiency and per unit of gain cost. Mehala & Moorthy, [36] announced that, the mean return over feed cost differs significantly between treatment groups which are mainly due to difference in feed cost of *Aloe vera* and *Curcuma longa* inclusion in broiler diet. Previous result indicated that dietary addition of *E. officinalis* (Amla) fruit powder at the rate of 0.4% and 0.8% had higher growth rate and net profit per bird in commercial broiler chickens [38].

Emblica officinalis supplementation was investigated on growth performance and economic returns for Vencobb-400 broilers by Gaikwad et al. [42]. Gaikwad et al. [42] asserted that *Emblica officinalis* powder resulted higher economic profit. It was suggested that supplementation of 1% *Emblica officinalis* powder can enhance economic returns without exhibiting any perceptible side effect [42]. It was disclosed that, the higher net profit per bird in *E. officinalis* supplemented groups attributed to higher body weights compared to the un-supplemented group and similar feed intakes among all the treatment groups [38]. Present research rumored that higher dose of MPC was potential in exhibiting profound body weight and body weight gain as well as better FCR suppression of pathogenic microbes and no profound impact on hematology [64-66]. The cost per unit of gain was better in case of MPC incorporated birds of the current study indicated better utilization of medicinal plant byproducts and better combination might be due to synergism of the phenolic compounds of the experimental materials *Camelia sinensis, Aloe vera* and *Phyllanthus emblica.*

CONCLUSION

Present experiment was conducted and designed with pharmacologically active medicinal byproduct combinations (*Camelia sinensis, Aloe vera* and *Phylanthus emblica*) as feed additives to investigate growth performance, fecal pH and microbiology, hematological parameters and economic efficacy in broiler chicken. Dietary treatments were: 1) MPC0=Control (basal diet without medicinal byproduct combinations); 2) MPC1 = basal diet + 0.2% medicinal byproduct combination; 3) MPC2 = basal diet + 0.4% medicinal byproduct combinations; 4) MPC3 = basal diet + 0.6% medicinal byproduct combination. The result of present study revealed that, medicinal byproduct combinations added groups exhibited better broiler growth performance; lower fecal pH and suppression of pathogenic *E. coli* was observed in MPC2 and MPC3 and *Salmonella sp.* was observed lower in MPC1, MPC2 and MPC3 supplemented birds relative to that of MPC0. Hematological data indicated that, there was no profound negative impact except the lower RBC count was found in MPC2 as compared to MPC0. The economic efficacy was better in case of broilers treated with MPC1, MPC2 and MPC3 compare to MPC0. Therefore, medicinal byproduct combinations with *Camelia sinensis, Aloe vera* and *Phylanthus emblica* could be a potential feed additives in case of broilers, where MPC2 could be preferred for better efficacy.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding publishing this article.

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