Global Veterinaria 12 (5): 627-635, 2014
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2014.12.05.83148

Effect of Turmeric (*Curcuma Longa*), Fenugreek (*Trigonella foenum-graecum L.*) And/or Bioflavonoid Supplementation to the Broiler Chicks Diet and Drinking Water on the Growth Performance and Intestinal Morphometeric Parameters

[1]H.A. Abdel-Rahman, [1]S.I. Fathallah, [2]M.A. Helal, [3]A.A. Nafeaa and [1]I.S. Zahran

[1]Department of Physiology, Faculty of Veterinary Medicine, Sadat City University, Sadat City, Egypt
[2]Department of Husbandry & Animal Wealth Development,
Faculty of Veterinary Medicine, Sadat City University, Sadat City, Egypt
[3]Department of Physiology, Faculty of Veterinary Medicine, Benha University, Egypt

Abstract: The present study was carried out to determine the effect of herbal mixture (fenugreek & curcumine) and/or bioflavonoid supplementation to the broiler diet and drinking water on growth performance and morphometeric study of intestine. Herbal mixture diet and Bioflavonoid watering significantly (P<0.05) affect live body weight (1675.38 and 2242.18, respectively) when compared with other groups at 4th and 5th week. Watering of Bio-Guard and Aqueous herbal extract was the highest relative body weight gain (0.28) and differed significantly (P<0.05) from control (0.18), but it has no significant (P<0.05) difference with other groups at 5th week, However, group 1 (0.98) recorded the higher significant value (P<0.05) when compared with control and those received herbal mixture in diet and watering of Bio-Guard and aqueous herbal extract. Birds received basic diet and watering of Bio-Guard and aqueous herbal extract (1.65) were differed significantly (P<0.05) in feed conversion from other groups. The supplementation of herbal mixture and /or bioflavonoids to broiler feed increased the villus height and width, crypt depth and surface area in treated groups in comparison with control group. Overall, the current study recommends use combination of bioflavonoid (Bio-Guard- in drinking water) and herbal mixture (fenugreek & curcumine mixed in ration) as in group 1. This synergistic effect of Bio-Guard and herbal mixture were reflected on the highest significant records of the live body weight, body weight gain, feed conversion ration and reduction of the marketing age (35 days) and rearing cost of broiler chicks.

Key words: Turmeric · Fenugreek · Bioflavonoids · Broiler · Growth Performance · Intestinal morphometery

INTRODUCTION

Antibiotics as growth promoter in poultry feed are posing serious health risks to human health, because of their residual effects in poultry meat and eggs result pathogens develop resistance to antibiotics. Poultry scientists today are challenged to find out new alternatives to antibiotic growth promoters with no side effects for poultry that could be more or as effective against harmful micro organisms in the gastrointestinal tract and to stimulate the growth by increasing the efficiency of feed utilization and to enhance the immunity. There are a lot number of compounds and products in nature that have the potential of stimulating growth and combating various diseases by the virtue of being antibacterial, antifungal etc. Phytobiotics are the substances obtained from the medicinal plants and herbs have wide range medicinal properties and are the best possible alternatives to antibiotics as growth promoter [1].

Fenugreek leaves and seeds have been used extensively to prepare extracts and powders for medicinal uses [2]. It's reported to have anti-diabetic, anti-fertility, anti-cancer, anti-microbial, antiparasitic, hypo-cholesterolaemic effects [3]. It also contains neurin, biotin, trimethylamine which tends to stimulate the appetite by their action on the nervous system [4]. Moreover, Fenugreek contains minerals, B-complex, iron,

Corresponding Author: Hassan A. Abdel-Rahman, Department of Physiology, Faculty of Veterinary Medicine, Sadat City University, Sadat City , Egypt.

phosphates, PABA (Para-Amino Benzoic Acid), vitamins (A, D), lecithin and choline that help to dissolve cholesterol and fatty substances [5].

Plants of the *Zingiberaceae* family have been widely used in dietary cuisines and in traditional oriental medications without any serious adverse reactions. Some phenolic substances present in Zingiberaceae plants generally possess strong anti-inflammatory and anti-oxidative properties and exert substantial anticarcinogenic and anti-mutagenic activities [6]. These plants also accumulate pharmacologically important active metabolites in their rhizomes at high levels [7]. Moreover, The main component of turmeric is curcumin that is a good antioxidant [8], nematocidal [9] and antibacterial [10]. Fortunately the safety of turmeric and its yellow coloring agent, curcumin, has been extensively used for imparting color and flavor to foods and also for the treatment of a variety of inflammatory conditions and other diseases which are approved by many organizations and researchers [11- 13].

Turmeric is a medicinal plant widely used and cultivated in tropical regions. Plant extracts were found to have antifungal [14], immunomodulatory [15], antioxidative [16] and antimutagenic [17] activities. Some of the pharmacological activities of turmeric as nematocidal [9] and anti-inflammatory [18] were demonstrated. Furthermore, the plant were used predominantly for endoparasites as well as internal and external injuries [19]. Moreover, Soni *et al*. [17] proved the protective effect of turmeric food activities on aflatoxin-induced mutagenicity and hepatocarcinogenicity.

Many researchers were used only fenugreek [1, 20, 21] or turmeric [22-24] in poultry production. So, these results urged us to evaluate the co-supplementation (synergistic) effects of fenugreek (*Trigonella foenum-graecum L.*) and turmeric (*Curcuma longa*) and/or polyherbal bioflavonoids mix (Bio-Guard) to the diet and water of broiler chickens on the growth performance and intestinal morphometeric parameters under practical conditions.

MATERIALS AND METHODS

Plant Materials: Dry fenugreek seeds *(Trigonella foenum-graecum L)* and turmeric rhizome (*Curcuma longa*) were obtained from local market of Cairo province, Egypt (December, 2012). The sample specimen was identified by a taxonomist of Botany Department, Faculty of Science, Alazhar University, Cairo Egypt. Samples of these plants were kept frozen for future reference.

Plant Aqueous Extracts Preparation and Administration: Fenugreek seed infusion was prepared according to the method described by Khan *et al.* [1] with some modification. Seed and rhizome were washed and dried for 24 hours at 37°C in incubator. The Dry seed and rhizome were then ground in a grinding machine; 100.0 g of dried ground fenugreek seed and 50.0 g of turmeric rhizome were taken in a non-metallic jar and half liter of hot boiled distilled water were poured on it and continuous mixing by heat-stage stirrer at 50°C for 5 hours to prepare an infusion. The macerate was filtered through double layered muslin cloth to yield 400 ml after filtration. The obtained aqueous extract was brownish yellow viscous residue. This volume of the extract was freshly prepared daily and added to the consumed drinking water/100 chicks/daily up to the end of the experimental period (32day).

Addition of Herbal Mixture to Ration : Fenugreek seed (5.33 kg) and turmeric rhizome (2.66 kg) per ton of finisher diet was prepared to be consumed by the tested group. The birds of the tested groups consumed this ration at day 16 of the experimental period (32 days).

Birds Housing and Grouping: Two hundreds and fifty, one-day-old, unsexed commercial broiler chicks (Cobb) were assigned into 5 groups of 50 chicks in a chamber at the 16th day of the experimental period (32d.). All experimental procedures used in the present study were approved by the Animal Care and Use Committee at the National Institute of Agricultural Science and Technology and conform to the US National Institutes of Health guidelines for the care and use of laboratory animals. All birds were vaccinated against Newcastle (ND) and infectious bronchitis (IB) strains and infectious bursal disease (Gumboro).

- Group 1 (Herbal mixture diet + *Bioflavonoid watering)- Birds received ration mixed with herbal mixture (Table 1) plus bioflavonoid (Bio-Guard) twice daily in drinking water.
- Group 2 (Control group)- Chicks fed only basic diet (Table 1) plus clear water (without BioGuard) supplementation).
- Group 3 (Basic Diet + Bio-Guard watering) - Birds received basic ration plus Bio

Guard Twice Daily All over the Experimental Period: Group 4 (Herbal mixture diet + Watering of Bio-Guard & Aqueous herbal extract)- Birds received ration mixed with

Table 1: The ingredients and composition of basal diet.

Ingredients and composition (%)	Starter	Finisher
Corn	55.59	61.07
Soy bean meal	37.32	31.83
Soy oil	2.98	3.41
Lime stone	1.21	1.42
Dicalcium Phosphate	1.60	1.16
DL. Methionine	0.20	0.10
*Vitamin and Minerals	0.60	0.60
Sodium chloride	0.23	0.18
Sodium bicarbonate	0.27	0.23
Chemical Analysis (%)		
ME (kcal/kg)	2950	3050
Crude Protein (%)	21.20	19.16
Lysine (%)	1.14	1.01
Methionine (%)	0.50	0.39
Methionine and Cysteine (%)	1.03	0.84
Available Methionine + Cysteine (%)	0.85	0.71
Calcium (%)	0.93	0.90
Available Phosphate (%)	0.44	0.35

*Supplied per kilogram of diet: vitamin A, 1,500 IU; cholecalciferol, 200 IU; vitamin E, 10 IU; riboflavin, 3.5 mg; pantothenic acid, 10 mg; niacin, 30 mg; cobalamin, 10 µg; choline chloride, 1,000 mg; biotin, 0.15 mg; folic acid, 0.5 mg; thiamine 1.5 mg; pyridoxine 3.0 mg; iron, 80 mg; zinc, 40 mg; manganese, 60 mg; iodine, 0.18 mg; copper, 8 mg; selenium, 0.15 mg.

herbal mixture plus Bio-Guard at the morning and aqueous herbal mixture extract mixed with water at the evening from the 16th to 32days of the experimental period.

- Group 5 (Basic Diet + Watering of Bio-Guard & Aqueous herbal extract)- Birds received classic ration without any treatment plus Bio-Guard at the morning and herbal mixture extract mixed with water at the evening from the 16th of the cycle till the remaining period.

***Bio-Guard:** [Egyptian Veterinary Products Company (EVEPCO)- Egypt]

***Acts as Antioxidant:** Bio-Guard is a comprehensive well balanced antioxidant formula of 100% plant origin. Each liter contains vitamin C [3.348 g], vitamin A [476.619 IU], zinc sulfate [14.035 g], sodium selenite [3.240 g] and total bioflavonoid content (Vitamin P ~5%) in a soluble active form.

***Dose:** 2.5ml/L of drinking water

Growth Performance Parameters: Feed intake (FI), body weight (BW), body weight gain (BWG), feed conversion ratio (FCR, g feed/ g gain) were recorded weekly for each group.

Intestinal Morphometeric Measurements: The entire small intestinal tract was removed for histomorphological examination. Two cm tissue samples of small intestine were taken from upper (start point of organ), middle (center point of organ) and lower (end point of organ) parts of duodenum (from the gizzard outlet to the end of the pancreatic loop), jejunum (from the pancreatic loop to Meckel's diverticulum) and ileum (from Meckel's diverticulum to the ileo-caeco-colic junction), according to the method of Giannenas *et al.* [25].

Briefly, the tissue samples were preserved in 10% neutral buffered formaldehyde (NBF) for 72 h, then processed for dehydration and clearing and embedded in wax. Histological sections (5µm thick) were prepared, fixed and stained with haemotoxylin and eosin. The tissue sections were examined on a Nikon phase contrast microscope coupled with a Micro computer integrated digital imaging analysis system (Nikon Eclipse 80i, Nikon Co. Tokyo, Japan). The height of 30 villi and the depth of 30 crypts were measured from each replicate per segment. The mean was obtained from these values. Villus height was measured from tip (with a lamina propria) of the villus to the base (villus-crypt junction) and villus width was measured at its middle part; while, the crypt depth was measured from villus-crypt junction to the distal limit of the crypt [26]. Finally, the values of villus height and villus width from six birds per group were expressed as mean villus height and villus width for one treatment group [27]. Surface area was calculated according to Sakamoto *et al.*[28] formula.

$$\text{Surface area} = (2\pi) \times (VW/2) \times (VL)$$

Where: VW=Villus width, VL= Villus length

Statistical Analysis: Results are expressed as mean ± standard error (SE). Differences between means in different groups were tested for significance using a one-way analysis of variance (ANOVA) followed by Duncan's test and *P* value of 0.05 or less was considered significant using the statistical analysis system, SPSS [29].

RESULTS

Growth Performance (Live Body Weight, Relative Weight Gain, Body Weight Gain and Feed Conversion Ratio): -The obtained data in Tables 2, 3, 4 and 5 revealed the following :

Table 2: Effect of herbal mixture and/or bioflavonoids supplementation to the broiler diet and drinking water on live body weight (g).

	Group				
Week	G1	G2	G3	G4	G5
W0	45.00±0.00[a]	45.00±0.00[a]	45.00±0.00[a]	45.00±0.00[a]	45.00±0.00[a]
W1	195.67±7.51[a]	200.33±6.96[a]	196.67±3.93[a]	200.00±1.53[a]	188.00±7.81[a]
W2	457.99±4.46[a]	452.34±5.02[a]	447.14±5.58[a]	449.17±7.05[a]	449.96±2.17[a]
W3	863.15±13.47[a]	836.50±40.33[a]	862.50±28.68[a]	828.45±24.85[a]	852.75±30.69[a]
W4	1675.38±8.74[a]	1570.17±33.38[bb]	1591.93±26.03[b]	1585.74±20.13[bb]	1505.48±41.48[b]
W5	2242.18±31.32[a]	2020.19±50.32[b]	2101.05±57.14[b]	2047.43±57.57[b]	2095.76±38.58[b]

Means ± SE

[A, b, c…]Means (In the same raw) having different superscripts are significantly different at $P < 0.05$. G1= Herbal mixture (in diet) + Bioflavonoids, G2= Basic diet (Control group), G3=Basic diet+ Bioflavonoids, G4= Herbal mixture (in diet) +Herbal mixture (in water) +Bioflavonoids, G5= Basic diet+ Herbal mixture (in water) +Bioflavonoids.

Table 3: Effect of herbal mixture and/or bioflavonoids supplementation to the broiler diet and drinking water on relative weight gain (RWG- %).

	Group				
Week	G1	G2	G3	G4	G5
W1	0.77 ±0.09[a]	0.77 ±0.08[a]	0.77 ±0.00[a]	0.77 ±0.00[a]	0.76 ±0.00[a]
W2	0.58 ±0.02[a]	0.55 ±0.02[a]	0.56 ±0.02[a]	0.55 ±0.01[a]	0.58 ±0.02[a]
W3	0.47 ±0.01[a]	0.46 ±0.03[a]	0.48 ±0.01[a]	0.47 ±0.02[a]	0.47 ±0.02[a]
W4	0.48 ±0.01[a]	0.49 ±0.03[a]	0.48 ±0.04[a]	0.47 ±0.02[a]	0.32 ±0.14[a]
W5	0.25 ±0.01[ab]	0.18 ±0.04[b]	0.22 ±0.03[ab]	0.22 ±0.03[ab]	0.28 ±0.02[a]
Total RWG	0.98 ±0.00[a]	0.98 ±0.00[b]	0.98 ±0.00[ab]	0.98 ±0.00[b]	0.98 ±0.00[ab]

Means± SE

[A, b, c]…Means (In the same raw) having different superscripts are significantly different at P < 0.05. G1= Herbal mixture (in diet) +Bioflavonoids, G2= Basic diet (Control group), G3=Basic diet+ Bioflavonoids, G4= Herbal mixture (in diet) +Herbal mixture (in water) +Bioflavonoids, G5= Basic diet+ Herbal mixture (in water) +Bioflavonoids.

Table 4: Effect of herbal mixture and/or bioflavonoids supplementation to the broiler diet and drinking water on body weight gain (BWG- g).

	Group				
Week	G1	G2	G3	G4	G5
W1	150.67±7.51[a]	155.33±6.96[a]	151.67±3.93[a]	155.00±1.53[a]	143.00±7.81[a]
W2	269.80±9.54[a]	243.95±9.05[a]	248.80±11.22[a]	245.47±3.43[a]	256.42±7.34[a]
W3	402.73±15.05[a]	389.79±43.51[a]	417.03±24.68[a]	388.47±25.16[a]	407.21±30.14[a]
W4	802.05±14.11[a]	803.50±43.59[a]	789.17±86.25[a]	731.55±44.79[a]	555.25±187.59[a]
W5	560.66±33.87[a]	400.43±59.38[a]	484.74±75.71[a]	467.43±67.84[a]	590.28±49.15[a]
Total BWG	2197.18±31.32[a]	1975.19±50.32[b]	2056.05±57.14[b]	2002.43±57.57[b]	2050.76±38.58[b]

Me[a]ns ±SE

[A, b, c]…Means (In the same raw) having different superscripts are significantly different at P < 0.05.

G1= Herbal mixture (in diet) +Bioflavonoids, G2= Basic diet (Control group), G3=Basic diet+ Bioflavonoids, G4= Herbal mixture (in diet) +Herbal mixture (in water) +Bioflavonoids, G5= Basic diet+ Herbal mixture (in water) +Bioflavonoids

Table 5: Effect of herbal mixture and/or bioflavonoids supplementation to the broiler diet and drinking water on feed conversion ratio (FC).

	Group				
Week	G1	G2	G3	G4	G5
W1	1.52±0.08[a]	1.47±0.07[a]	1.50±0.04[a]	1.47±0.01[a]	1.60±0.09[a]
W2	1.59±0.06[a]	1.77±0.06[a]	1.74±0.08[a]	1.75±0.02[a]	1.68±0.05[a]
W3	2.63±0.10[a]	2.64±0.35[a]	2.17±0.13[a]	2.49±0.18[a]	2.14±0.18[a]
W4	1.86±0.03[a]	1.51±0.09[a]	1.72±0.19[a]	2.03±0.14[a]	1.5±0.14[a]
W5	1.86±0.11[b]	2.63±0.66[ab]	2.27±0.86[ab]	3.74±0.84[a]	1.59±0.14[b]
Total FC	1.89±0.03[ab]	1.95±0.08[ab]	1.85±0.05[b]	2.03±0.0520[a]	1.65±0.03[b]

Means ± SE

[A, b, c]…Means (In the same raw) having different superscripts are significantly different at P < 0.05. G1= Herbal mixture (in diet) +Bioflavonoids, G2= Basic diet (Control group), G3=Basic diet+ Bioflavonoids, G4= Herbal mixture (in diet) +Herbal mixture (in water) +Bioflavonoids, G5= Basic diet+ Herbal mixture (in water) +Bioflavonoids.

- Live body weight recorded significant difference (P<0.05) in Group1 when compared with other groups at fourth and fifth week.
- Relative body weight gain of G5 was the highest value and was differed significantly (P<0.05) from G2, but it has no significant (P<0.05) difference with other groups at fifth week, However G1 has the higher significant value (P<0.05) when compared with G2 and G4 at total relative body weight gain.
- Body weight gain of G1 was differed significantly (P<0.05) from other groups at the end of the experiment.
- Feed conversion ratio of G4 and G1 was differed significantly (P<0.05) from other groups.

Morphometeric Studies of Broiler Intestine.

Duodenum Micro-morphological Measurements: The obtained data in Table 6 illustrated the following:

- A significant difference (P< 0.05) in G1, G3 and G4 relative to G2 in villous height of duodenum.
- A significant difference (P< 0.05) between G3 and G1, G2, G4 and G5 in duodenal villous width.
- A significant difference (P< 0.05) in G1, G3, G4 and G5 relative to G2 in duodenal crypt depth.
- A significant difference (P< 0.05) in G5 relative to G1, G2, G3 and G4 in duodenal musclosa.
- A significant difference (P< 0.05) in G3 relative to G1, G2, G4 and G5 in duodenal surface area.

Jejunum Micro-morphological Measurements: The obtained data in Table 7 demonstrated the following:

- A non significant difference between all poultry groups in villous height, width and surface area of jejunum.
- A significant difference (P< 0.05) in G2 relative to G4 in jejunal crypt depth.
- A significant difference (P< 0.05) in G5 relative to G1, G2, G3 and G4 in jejunal musclosa.

Ileum Micro-morphological Measurements: The obtained data in Table 8 revealed the following:

- A significant difference (P< 0.05) in G4 relative to G1 in villous height of ileum.
- A significant difference (P< 0.05) between G1 and G2, G3 and G5 in ileum villous width.
- A significant difference (P< 0.05) in G3 relative to G1, G2, G4 and G5 in ileum crypt depth.

Table 5: Effect of herbal mixture and/or bioflavonoids supplementation to the broiler diet and drinking water on feed conversion ratio (FC).

	Group				
Week	G1	G2	G3	G4	G5
W1	1.52±0.08[a]	1.47±0.07[a]	1.50±0.04[a]	1.47±0.01[a]	1.60±0.09[a]
W2	1.59±0.06[a]	1.77±0.06[a]	1.74±0.08[a]	1.75±0.02[a]	1.68±0.05[a]
W3	2.63±0.10[a]	2.64±0.35[a]	2.17±0.13[a]	2.49±0.18[a]	2.14±0.18[a]
W4	1.86±0.03[a]	1.51±0.09[a]	1.72±0.19[a]	2.03±0.14[a]	1.5±0.14[a]
W5	1.86±0.11[b]	2.63±0.66[ab]	2.27±0.86[ab]	3.74±0.84[a]	1.59±0.14[b]
Total FC	1.89±0.03[ab]	1.95±0.08[ab]	1.85±0.05[b]	2.03±0.0520[a]	1.65±0.03[b]

Means ± SE

[A, b, c]…Means (In the same raw) having different superscripts are significantly different at P < 0.05. G1= Herbal mixture (in diet) +Bioflavonoids, G2= Basic diet (Control group), G3=Basic diet+ Bioflavonoids, G4= Herbal mixture (in diet) +Herbal mixture (in water) +Bioflavonoids, G5= Basic diet+ Herbal mixture (in water) +Bioflavonoids.

Table 6: Effect of herbal mixture and/or bioflavonoids supplementation to the broiler diet and drinking water on duodenum micro-morphological measurements.

	Groups				
Parameters	G1	G2	G3	G4	G5
Villous height (µ)	1200.00±45.00[a]	1000.00±42.00[b]	1180.00±48.00[a]	1200.00±36.00[a]	1090.00±49.00[ab]
Villous width (µ)	180.00±10.00[b]	200.00±7.00[b]	330.00±21.00[a]	230.00±39.00[b]	180.00±8.00[b]
Crypt depth (µ)	180.00±7.00[a]	140.00±5.00[b]	190.00±7.00[a]	180.00±9.00[a]	190.00±7.00[a]
Musclosa (µ)	180.00±10.00[b]	190.00±12.00[b]	190.00±12.00[b]	190.00±7.00[b]	230.00±12.00[a]
Surface area (mm^2)	676.22±45.45[b]	626.70±30.78[b]	1262.02±123.17[a]	864.54±153.42[b]	617.10±49.44[b]

[a, b, c]…Means (In the same raw) having different superscripts are significantly different at P < 0.05. G1= Herbal mixture (in diet) +Bioflavonoids, G2= Basic diet (Control group), G3=Basic diet+ Bioflavonoids, G4= Herbal mixture (in diet) +Herbal mixture (in water) +Bioflavonoids, G5= Basic diet+ Herbal mixture (in water) +Bioflavonoids.

Table 7: Effect of herbal mixture and/or bioflavonoids supplementation to the broiler diet and drinking water on jejunum micro-morphological measurements.

	Groups				
Parameters	G1	G2	G3	G4	G5
Villous height (µ)	1240.00±6.00[a]	1250.00±4.00[a]	1200.00±7.00[a]	1160.00±6.00[a]	1210.00±6.00[a]
Villous width (µ)	200.00±50.00[a]	190.00±10.00[a]	230.00±13.00[a]	190.00±7.00[a]	200.00±12.00[a]
Crypt depth (µ)	170.00±8.00[ab]	190.00±11.00[a]	170.00±4.00[ab]	160.00±9.00[b]	170.00±8.00[ab]
Musclosa (µ)	180.00±7.00[b]	160.00±17.00[b]	180.00±9.00[b]	170.00±9.00[b]	230.00±15.00[a]
Surface area (mm)	717.33±140.04[a]	741.77±50.01[a]	889.26±88.39[a]	680.49±42.98[a]	779.03±72.75[a]

[a, b, c]…Means (In the same raw) having different superscripts are significantly different at P < 0.05. G1= Herbal mixture (in diet) +Bioflavonoids, G2= Basic diet (Control group), G3=Basic diet+ Bioflavonoids, G4= Herbal mixture (in diet) +Herbal mixture (in water) +Bioflavonoids, G5= Basic diet+ Herbal mixture (in water) +Bioflavonoids.

Table 8: Effect of herbal mixture and/or bioflavonoids supplementation to the broiler diet and drinking water on ileum micro-morphological measurements.

	Groups				
Parameters	G1	G2	G3	G4	G5
Villous height (µ)	690.00±23.00[b]	790.00±44.00[ab]	700.00±23.00[bb]	840.00±25.00[a]	720.00±34.00[bb]
Villous width (µ)	220.00±9.00[a]	150.00±6.00[b]	180.00±9.00[b]	170.00±7.00[bb]	150.00±9.00[b]
Crypt depth(µ)	160.00±0.008[b]	150.00±0.01[b]	180.00±0.01[a]	140.00±0.01[b]	120.00±0.01[b]
Musclosa (µ)	190.00±6.00[bb]	160 .00±9.00[b]	400 .00±38.00a	250.00±12.00[b]	240.00±9.00[bb]
Surface area (mm)	469.91±27.75[a]	375.65±21.14[bb]	393.57±25.25[b]	439.76±17.67[ab]	326.58±18.85[b]

[a, b, c]…Means (In the same raw) having different superscripts are significantly different at P < 0.05. G1= Herbal mixture (in diet) +Bioflavonoids, G2= Basic diet (Control group), G3=Basic diet+ Bioflavonoids, G4= Herbal mixture (in diet) +Herbal mixture (in water) +Bioflavonoids, G5= Basic diet+ Herbal mixture (in water) +Bioflavonoids.

Table 9: Effect of herbal mixture and/or bioflavonoids supplementation to the broiler diet and drinking water on intestine macro-morphological measurements (cm).

	Groups				
Length	G 1	G 2	G 3	G 4	G 5
Duodenal	25.00±0.89[a]	24.67±0.99[a]	25.67±0.84[a]	25.00±1.24[a]	25.00±1.07[a]
Lower intestine	182.00±5.03[a]	168.00±4.53[ab]	179.50±6.20[a]	160.67±5.72[b]	160.33±7.68[b]
Cecum	18.08±0.90[a]	18.17±0.76[a]	18.08±0.55[a]	17.58±0.80[a]	17.17±0.48[a]

[a, b, c]…Means (In the same raw) having different superscripts are significantly different at P < 0.05. G1= Herbal mixture (in diet) +Bioflavonoids, G2= Basic diet (Control group), G3=Basic diet+ Bioflavonoids, G4= Herbal mixture (in diet) +Herbal mixture (in water) +Bioflavonoids, G5= Basic diet+ Herbal mixture (in water) +Bioflavonoids.

- A significant difference (P< 0.05) in G5 relative to G1, G2, G3 and G4 in ileum musclosa.
- A significant difference (P< 0.05) in G1 relative to G3 and G5 in ileum surface area.

Intestinal Macro-morphological Measurements: The obtained data in Table 9 demonstrated the following:

- A non significant difference between all poultry groups in duodenal length and right cecum length.
- A significant difference (P< 0.05) in G1 and G3 relative to G4 and G5 in lower intestine length.

DISCUSSION

The present study declared that adding herbal mixture (turmeric & fenugreek in diet) and Bioflavonoids (in water) recorded significant effect (P<0.05) on live body weight when compared with other groups at fourth and fifth week. These findings were in harmony with Murray *et al*. [30] and Hernandez *et al.* [31] results, who stated that the improvement in body weight may be due to the presence of the fatty acids or due to stimulating effect of the digestive system of broilers. Also Azoua [32] was noted that adding fenugreek to broiler diet resulted in an increased body weight.

The findings of the current study were contradictory to Abbas [20] conclusion who stated that insignificant effect on body weight of birds consuming fenugreek seeds relative to control diets.

The present study revealed that herbal mixture in diet and Bioflavonoid watering had the highest values (P<0.05) of relative weight gain comparable to other groups. These results may

be due to increase existence of some extracts such as phenolic acids, which act as antimicrobial and cause sterilization of the gastrointestinal tract, so as a result improved feed utilization and result in increasing weight gains [33, 34].

The present study revealed that group 5 possess significant difference (P<0.05) relative to control group. This result harmonized with Alloui *et al.* [35] who stated that the fenugreek seeds significantly (p<0.05) affected feed conversion ratio. This effect is related to the development of the broiler chick's gut. Morphological changes of gastrointestinal tissues can be induced by differences in gut load of microbial content including their metabolites [36].

Also these results agree with the finding of El-Gendi *et al.* [37] who indicated that there was an improvement in feed conversion with feeding herbal products as feed additives that could be attributed to their effect on improving the digestibility of dietary protein in the small intestine.

The present results revealed that the supplementation of herbal mixture and /or bioflavonoids to broiler feed increased the villus height and width, crypt depth and surface area in treated groups in comparison with control group.

Our results were matched with Pluske *et al.* [38]; Samanya and Yamauchi [39]. This outcome might be due to increased epithelial cell turnover in all segments (duodenum, jejunum and ileum) of small intestine. Also, longer villi are associated with activated cell mitosis. Longer villi provided more nutrients absorption area in small intestine, which might enhance nutrient absorption. Although, short or damaged intestinal villi impair the absorption capability of animals or birds due to decreased absorption area of intestine, which might lead to poor feed efficiency and weight gain [40].

In contrast, shortening of villi with deeper crypts may lead to poor nutrient absorption and lower performance [36]. The intestinal epithelial cells originate in the crypt and migrate along the villus surface upward to the villus tip [41]. Deeper crypts indicate fast tissue turnover to permit renewal of the villus as needed in response to tissue sloughing, inflammation or toxins produced by any pathogen and high demands for tissue [42].

Furthermore, the finding of this study revealed that duodenal length of treated groups have significant difference with control group and this matched with Miles [43] findings. Growth promoters are known to inhibit normal early microbial proliferation and hence competition for needed nutrients during the gut maturation process. Moreover, increase gastro-intestinal surface area leads to changes in GIT morphology as well as decreased cell proliferation, thinner mucosa and less lamina propria [44].

In conclusion, the fore mentioned results confirm the beneficial use of fenugreek, curcumine as feed additives and bioflavonoid (Bio-Guard) in water in broiler production, as it increases production performances. Also this mixture can be an alternative to antibiotic growth promoters and is highly recommended as feed supplement. It may also decrease the marketing age of broilers and reduce their rearing cost (35 days). Overall, the current study recommends use combination of bioflavonoid (Bio-Guard- in drinking water) and herbal mixture (fenugreek & curcumine mixed in ration) as in group 1. This synergistic effect of Bio-Guard and herbal mixture were reflected on the highest significant records of the live body weight, body weight gain and feed conversion ration of broiler chicks.

REFERENCES

1. Khan, F.U., F.R. Durrani, A. Sultan, R.U. Khan and S. Naz, 2009. Effect of fenugreek (*trigonella foenum-graecum*) seed extract on visceral organs of broiler chicks. ARPN Journal of Agricultural and Biological Science, 4(1): 58-60.
2. Basch, E., C. Ulbricht, G. Kuo, P. Szapary and M. Smith, 2003. Therapeutic applications of fenugreek. Alt. Med. Rev., 8: 20-27.
3. Al-Habori, M. and A. Roman, 2002. Pharmacological properties in fenugreek-The genus *Trigonella*. 1st Edn. by G.A. Petropoulos (Ed.). Taylor and Francis, London and New York, 10: 163-182.
4. Michael, D. and D. Kumawat, 2003. Legend and archeology of fenugreek, constitutions and modern applications of fenugreek seeds. International Symp. USA. pp: 41-42.
5. Dixit, P., S. Ghaskadbi, H. Mohan and T.P. Devasagayam, 2005. Antioxidant properties of germinated fenugreek seeds. Phytotherapy Research, 19: 977-983.
6. Lee, E. and Y. J. Surh, 1998. Induction of Apoptosis in HL-60 Cells by Pungent London and New York, 10: 163-182.
7. Ahumada, M.D.C.R., B.N. Timmermann and D.R. Gang, 2006. Biosynthesis of curcuminoids and gingerols in turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*): identification of curcuminoid synthase and hydroxycinnamoyl-CoA thioesterases. Phytochemistry, 67(18): 2017-2029.

8. Karami, M., A.R. Alimon, A.Q. Sazili, Y.M. Goh and M. Ivan, 2011. Effects of dietary antioxidants on the quality, fatty acid profile and lipid oxidation of longissimus muscle in Kacang goat with aging time. Meat Sci., 88: 102-108.
9. Kiuchi, F., Y. Goto, N. Sugimoto, N. Akao, K. Kondo and Y. Tusda, 1993. Nematocidal activity of turmeric: synergestic action of curcuminoids. Chem. Pharm Bull (Tokyo). 41: 1640-1643.
10. Negi, P.S., G.K. Jayaprakasha, L. Jagan Rao Mohan and K.K. Sakariah, 1999. Antibacterial activity of turmeric oil: a byproduct from curcumin. J. Agric. Food Chem., 47: 4297-4300.
11. WHO, 1987. Principles for the Safety Assessment of Food Additives and Contaminants in Food, Environmental Health Criteria, Vol. 174 p, 70. World Health Organization, Geneva.
12. Hallagan, J.B., D.C. Allen and J. Borzelleca, 1995. The Safety and Regulatory Status of Food, Drugs and Cosmetics, Colors Additives Exempt from Certification. Food Chem. Toxicol., 33(6): 515-528.
13. Deshpande, S.S., V.S. Lalitha, A.D. Ingle, A.S. Raste, S. G. Gadre and G. B. Maru, 1998. Subchronic Oral Toxicity of Turmeric and Ethanolic Turmeric Extract in Female Mice and Rats. Toxicol. Let., 95(3): 183-193.
14. Wuthi-udomler, M., W. Grisanapan, O. Luanratana and W. Caichompoo, 2000. South east Asian J. Trop Med. Public Health, 31(1): 178-182.
15. Antony, S., R. Kuttan and G. Kuttan, 1999: Immunomodulatory activity of curcumin. Immunol. Invest., 28: 291-303.
16. Osawa, T., Y. Sugiyama, M. Inayoshi and S. Kawakishi, 1995. Antioxidative activity of tetrahydrocurcuminoids. Biosci. Biotechnol Biochem. 59: 1609-1612.
17. Soni, K.B., M. Lahiri, P. Chackradeo, S.V. Bhide and R. Kuttan, 1997. Protective effect of food additives on aflatoxin-induced mutagenicity and hepatocarcinogenicity. Cancer letters, 115: 129-33.
18. Ammon, H.P., H. Safayhi, T. Mack and J. Sabieraj, 1993. Mechanism of anti inflammatory actions of curcumine and boswellic acids. J. Ethnopharmacol, 38: 113-119.
19. Lans, C. and G. Brown. 1998. Ethnoveterinary medicines used for ruminants in Trinidad and Tobago. Prev. Vet. Med., 35: 149-163.
20. Abbas, R.J., 2010. Effect of using fenugreek, parsley and sweet basil seeds as feed additives on the performance of broiler chickens. International Journal of Poultry Science, 9(3): 278-282.
21. Khan, F.U., A. Ullah, S. Sajid-ur-Rehman Naz and N. Rana, 2011. Fenugreek (*Trigonella foenum-graecum L.*) effect on muscle growth of broiler chicks. Research Opinions in Animal & Veterinary Sciences, 1(1): 1-3.
2. AL-Sultan S.I. 2003. The effect of *curcuma longa* (tumeric) on overall performance of broiler chickens. International Journal of Poultry Science, 2(5): 351-353.
23. AL-Sultan S.I. and A.A. Gameel, 2004. Histopathological changes in the livers of broiler chicken supplemented with turmeric (*curcuma longa*). International Journal of Poultry Science, 3(5): 333-336.
24. Malekizadeh, M., M.M. Moeini and S.H. Ghazi, 2012. The effects of different levels of ginger (*Zingiber officinale* Rosc) and Turmeric (*Curcuma longa* Linn) Rhizomes powder on some blood metabolites and production performance characteristics of laying hens. J. Agr. Sci. Tech., 14: 127-134.
25. Giannenas, D.T., E. Tsalie, E.F. Chronis, D. Doukas and D.T. Kyriazakis, 2010. Influence of dietary mushroom Agaricus bisporus on intestinal morphology and microflora composition in broiler chickens Research in Veterinary Science, 89: 78-84.
26. Aptekmann, K.P., S.M. Baraldi Arton, M.A. Stefanini and M.A. Orsi, 2001. Morphometric analysis of the intestine of domestic quails (Coturnix coturnix japonica) treated with different levels of dietary calcium. Anat. Histol. Embryol., 30: 277-280.
27. Yamauchi, K., J. Buwjoom, K. Koge and T. Ebashi, 2006. Histological alterations of the intestinal villi in chickens refed dietary sugar cane extract. Poultry Science, 85: 645-651.
28. Sakamoto, K., H. Hirose, A. Onizuka, M. Hayashi, N. Futamura, Y. Kawamura and T. Ezaki. 2000. Quantitative study of changes in intestinal morphology and mucus gel on total parenteral nutrition in rats. J. Surg. Res., 94: 99-106.
29. SPSS, 2004. Statistical Package for Social Sciences. Release 16.0.1 version. SPSS Inc.
30. Murray, R.K., D.K. Granner, P.A. Mayes, V.W. Rodwell, 1991. The Text Book of Harpers Biochemistry. 22 Ed. Appleton and large. Norwalk, Connecticut/Loss Altos, California.
31. Hernandez, A.I., J. Madrid, V. Garcia, J.E.T. Orengo, M.D. Meglas 2004. Influence of two plant extracts on broiler performances, digestibility and digestive organs size. Poultry Science, 83: 169-174.

32. Azoua, H.M., 2001. Effect of hot pepper and fenugreek seeds supplementation on broiler diets. PhD Thesis, Faculty of Agriculture, Alexandria University, Egypt.
33. Yoshino, K., I. Tomito, M. Sano, I. Oguni, H. Hara and M. Nakano, 1996. Effects of long term dietary supplementation on lipid peroxide levels in rats. Agric., 17: 79-85.
34. Abdel-Rahman, H.A. and A.A. Nafea, 2014. Effect of two probiotics and bioflavonoids supplementation to diet and drinking water on intestinal microflora and some immunological parameters (unpublished).
35. Alloui, N., S. Ben Aksa, M.N. Alloui and F. Ibrir. 2012. Utilization of Fenugreek (Trigonella Foenum-Graecum) as Growth Promoter for Broiler Chickens. J. World's Poult. Res., 2(2): 25-27.
36. Xu, Z.R., C.H. Hu, M.S. Xia, X.A. Zhan and M.Q. Wang. 2003. Effects of dietary fructo-oligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult. Sci., 82(6): 1030-1036.
37. El-Gendi, G.M., F.A. Ismail and S.M. El-Aggoury, 1994. Antidiabetic effects of fenugreek extract (Trigonella foenum-graecum L.) on domestic animals with special reference to carbohydrate metabolism. Journal of Ecotoxicology and Environmental Monitoring, 8: 103-108.
38. Pluske, J.R., M.J. Thompson, C.S. Atwood, P.H. Bird, I.H. Williams and P.E. Hartmann, 1996. Maintenance of villus height and crypt depth and enhancement of disaccharide digestion and monosaccharide absorption, in piglets fed on cow's whole milk after weaning. British Journal of Nutrition, 76: 409-422.
39. Samanya, M. and K. Yamauchi, 2002. Histological alterations of intestinal villi in chickens fed dried Bacillus subtilis var. natto. Comparative Biochemistry and Physiology, 133: 95-104.
40. Ilsley, S.E., H.M. Miller and C. Kamel 2005. Effects of dietary quillaja Saponin and curcumin on the performance and immune status of weaned piglets. Journal Animal Science, 83: 82-88.
41. Potten, C.S., 1998. Stem cells in the gastrointestinal epithelium: numbers, characteristics and death. Philosophical Transactions of the Royal Society of London Series B: Biological Sciences, 353: 821-830.
42. Yason, C.V., B.A. Summers and K.A. Schat, 1987. Pathogenesis of rotavirus infection in various age groups of chickens and turkeys pathology. American Journal of Veterinary Research, 48: 927-938.
43. Miles, R.D., 1985. The impact of virginiamycin on nutrient utilization. Pages 5-40 in Proc. Pacesetter Conf. Stafac (virginiamycin) Broilers. SmithKline Animal Health Products, West Chester, PA.
44. Geyra, A., Z. Uni and D. Sklan, 2001. Enterocyte dynamics and mucosal development in the post hatch chick. Poult. Sci., 80: 776-782.