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Original Article

Effects of Commercial Herbal and Chemical Medicines on Performance, GI Microbial Population, Intestinal Morphology and Serum Lipids of Broiler Chickens Challenged with Infectious Bronchitis Vaccine Virus

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Abstract

The aim of this study was to investigate the effect of commercial herbal and chemical medicines on growth performance, serum lipids, intestinal selected bacterial population and intestinal morphology of broiler chickens. In this study, 450 day-old female broiler chickens (Arian strain) were divided into 10 treatments with three replicates of 15 chicks per replicate. On day 14 of the experiment, birds in following treatments: 1) Anzofin®; 2) Antibiofin®; 3) Immunofin®; 4)Broncofin® ; 5) Zagrol®; 6) Mentofin®; 7) Enrofloxacin®;8) Bromhexin®; and 9) positive control received IB–4/91vaccine 5 times greater than the standard dose, but chickens in 10) negative control (NC) group was vaccinated with standard dose of IB vaccine. The birds in treatments 1 to 6 received herbal medicines in drinking water from days 15 to 48. Chickens in treatments 7 and 8 received Enrofloxacin® and Bromhexin®, from days 15 to 19 in drinking water. The highest feed intake, body weight, and body weight gain were observed in Bromhexin® treatment. The lowest body weight, body weight gain and highest FCR were observed in Zagrol® treatment. Immunofin® had the lowest FCR among all treatments. The highest and lowest European Production Efficiency Factor was observed in Immunofin® and Positive control group, respectively (P>0.05). Bacterial population in GI tract was reduced in Mentofin® treatment. Bromhexin® insignificantly improved villi height of duodenum, jejunum and ileum. The highest crypt depth in duodenum, jejunum and ileum was observed in Zagrol® treatment.

Key words: Chemical medicine, Plant medicine, Performance, Broilers, Infectious bronchitis

Introduction

Different kinds of products have been used to increase livestock growth for years. Growth promoters are feed additives that improve the rate and uniformity of growth as well as enhance the feed conversion ratio [1]. Growth promoting feed additives has a positive influence on the microbial ecosystem of gastrointestinal (GI) tract of animals which relieves the host animals from immune defense stress in critical situations and also increases the intestinal availability of essential nutrients for absorption, thereby helping animals to grow better within the framework of their genetic potential [2]. The use of antibiotics considering as growth promoters in animal production has become virtually universal. In fact, due to antibacterial activity, antibiotics can cause desirable effects by: 1) reducing the prevalence and severity of subclinical infections [3,4]; 2) reducing microbial use of nutrients [5]; 3) increasing absorption of nutrients due to thinning the intestinal wall; and 4) reducing the amount of growth-depressing metabolites produced by Gram-positive bacteria [6,7]. However, the possibility of developing resistant populations of bacteria and the residual effects of using antibiotics as growth promoters such as an allergy in farm animals have been led to the European Union and United States ban on the use of antibiotics on farm animals as feed additives [8]. There are some studies which suggest

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that there is a link between the agricultural use of antibiotics and antibiotic-resistant human infections [9,10]. Antibiotic- free food production requires access to new approaches in poultry nutrition. In providing such guidelines, health, general problems of nutrition, welfare of the birds, and finally environmental concerns about breeding poultry, must be considered. The last two decades have seen a substantial increase in the use of aromatic herbs and essential oils as feed additives in animal nutrition [11]. Compared with synthetic antibiotic and inorganic chemicals, plant derived products have proven to be natural, less toxic, residue free and thought to be ideal feed additive in feed animal production [12]. Phytobiotics increase the productivity performance of animals and the quality of products obtained from them through improving the properties of the feed [2] and cause gastrointestinal stimulation through sight and olfactory sense in order to be prepared for ingestion food and stimulate secretion of the digestive enzyme and the movements of the gastric [13,14]. For example, a significant increase was reported in the activation of pancreatic amylase, maltase and trypsin in broiler chicks that were fed with commercial essential oils [15].The majority of experimental results indicates reduced feed intake at largely unchanged body weight gain or final body weight, leading to an improved feed conversion ratio when feeding Essential Oils [2]. Feed herbal supplements can also directly and indirectly affect the microbial population of GI [16]. The antimicrobial activity has been known as the most important advantage of using essential oils [17]. These compounds have useful effects on the microbial ecosystem of GI through the control of pathogens [18]. In addition to the antibacterial properties of essential oils they also include hypolipidemic [19],‹antioxidant [20,21] and improving digestion properties [22]. The most important thing is that because of the mechanism affecting simultaneously on several targets, any specific resistance or the accustomed manner essential oils hasn't been reported so far [23].

Present experiment was planned to study the effects of herbal medicines including: Anzofin®, Antibiofin®, Immunofin®, Broncofin®, Zagrol®, and Mentofin®, with chemical medicines including: Enrofloxacin®, and Bromhexin®, on performance, GI microbial population, intestinal morphology and serum lipids of broilers in challenge with infectious bronchitis vaccine virus.

Materials and Methods

Experimental Animals and Design

Four hundred and fifty-day old female broiler chickens (Arian) were divided into 10 experimental groups with three replicates and 15 chicks per replicate. Treatments were: 1) Anzofin®(Az); 2) Antibiofin®(Ab); 3) Immunofin® (Im); 4) Broncofin® (Bk); 5) Zagrol®(Zg); 6) Mentofin® (Me); 7) Enrofloxacin®(Ef); 8) Bromhexin®(Bh); 9) Positive control (PC); and 10) Negative control (NC). On day 14, treatments 1 to 9 were challenged with IB- 4/91vaccine 5 times greater than the standard dose, but the negative control group received the standard dose of IB vaccine via eye drop. From day 15 until day 48, treatments 1 to 6 were received herbal medicines via drinking water. Treatments 7 and 8 were received chemical medicines from day 15 to 19. All treatments except Mentofin®, received experimental chemical and herbal medicines of 1 liter per 1000 liters via drinking water and Mentofin® was provided as 250 milliliter per 1000 liters. Treatments 9 and 10 didn't receive any medication.

The main compound of Anzofin® is Eucalyptus with some other medicinal plants. Antibiofin® contains the active ingredient of *Thymus vulgaris* and some other herbs, Broncofin® is a mixture of the active ingredients of some medicinal plants and the most important is Eucalyptus, Immunofin ® contains active ingredient of Echinacea and few other medicinal plants. Mentofin® constitutes of *Eucalyptus* and peppermint essential oils [24]. and Zagrol is essential oils of *Satureja khuzistanica* [25]. The commercial herbal medicines including: Anzofin®, Antibiofin®, Immunofin®, Broncofin® (Pars Imen Co., Tehran) and Zagrol (Khoraman Pharmaceutical Co., Lorestan) were purchased. All of the medicines were used according to the guidelines of the respective companies.

At the beginning of the experiment the chicks were weighed and randomly distributed into 10 treatment groups. The ingredients and composition of the basal diet (starter from 1 to 14, grower from 15 to 28 and finisher from 29 to 48 days of age) are presented in Table 1. All birds employed in the experiment were fed according to applicable recommendations of the National Research Council [26]. The birds were housed on floor pens. Feed and water were provided ad libitum throughout the experiment. Lighting schedule was 23L/1D. The temperature was gradually reduced from 32 °C by 3 °C in each week.

Table 1 Ingredients composition and chemical analysis of the basal diets (%)

Ingredients	1-14 d	15-28 d	29-48 d
Maize	49.82	52.11	47.09
Soybean meal (48)	41.08	35.03	30.96
Wheat	4.20	8.09	14.68
Soybean oil	1.10	1.29	4.23
Dicalcium phosphate	2.46	2.20	2.06
DL-methionine (980 g/kg)	0.34	0.26	0.16
L-lysine (980 g/kg)	0.23	0.19	0.03
Vitamin permix[a]	0.25	0.25	0.25
Mineral permix[b]	0.25	0.25	0.25
Limestone	-	0.05	-
Salt	0.27	0.28	0.28
Calculated analysis			
ME (Mj/Kg)	11.80	12.35	12.74
Crude protein	21.53	18.85	18.01
Ether extract	4.04	5.05	6.57
Calcium	0.88	0.92	0.95
Available P	0.44	0.45	0.47
Lysine	1.18	1.12	1.08
Methionine	0.38	0.37	0.36
Methionine + Cystine	0.90	0.82	0.72
Tryptophan	0.98	0.91	0.87

[a] Supplied the following per kilogram of diet: vitamin A, 8,000 IU; vitamin D3 (cholecalciferol), 3,000 IU; vitamin E (DL-alpha-tocopherylacetate), 25 IU; menadione, 1.5 mg ; vitamin B_{12} (cyanocobalamin), 0.02 mg; biotin, 0.1 mg; folacin (folic acid), 1 mg; niacin (nicotinic acid), 50 mg; pantothenic acid, 15 mg; pyridoxine (pyridoxineHCl), 4 mg; riboflavin, 10 mg; thiamin, 3 mg (thiamin mononitrate).

[b] Supplied the following per kilogram of diet: 10 mg of copper ($CuSO_4$); 1.0 mg of iodine Ca (IO_3) 2; 80 mg of iron ($FeSO_4\_H_2O$); 100 mg of manganese ($MnSO_4\_H_2O$); 0.15mg of selenium ($NaSeO_3$); 80 mg of zinc ($ZnSO_4\_H_2O$); and 0.5 mg of cobalt ($CoSO_4$).

Performance Data

Feed intake (FI), body weight (BW), body weight gain (BWG) and FCR were measured weekly throughout the experiment. Two birds from each replicate were randomly selected and sacrificed at 48 days of age. Each replicate was considered as an experimental unit in order to analyze the experimental performance data. The experiment was approved in animal care committee of the Tarbiat Modares University in Iran.

European Production Efficiency Factor (EPEF)

EPEF was calculated according to following equation [27]:

EPEF= Livability% × Live weight (Kg)/age (day) × FCR × 100

Microbial Sampling and Incubation

On day 42 of the experiment, two birds from each replicate were sacrificed via CO_2 inhalation. One gram of the ileo-cecal contents was collected aseptically. The contents were gently placed in sterile sampling tubes and immediately transferred on ice to the laboratory for microbial study. Serial dilutions (10−4 to 10−7) were made and Selective media of Plate Count Agar (Merck, Germany), De Man Rogosa Sharpe Agar (MRS) (Merck, Germany) and MacConkey Agar (E-Merck, Germany) were used for total aerobics; lactic acid bacteria and, coliforms respectively. Microbial populations for total aerobics and coliforms were counted after aerobic incubation at 37 °C for 24 h and lactic acid bacteria after aerobic incubation at 37 °C for 48 h [28].

Intestinal Morphology Assay

At 48 days of age middle sections (3-4 cm) of duodenum, jejunum and ileum of two birds from each replicate were cut and prepared for histological indices assay. The histological indices were measured according to Iji et al. [29] method. Intestinal tissue samples were fixed in formalin and dehydrated, cleared, and impregnated with paraffin. The processed tissue was then embedded in paraffin wax. The sections were cut (6 μm) from the waxed tissue on LEICA RM 2145 microtome, cleared of wrinkles by floating on warm water (55- 60 ºC) prior

to mounting on 10% poly-L-lysine coated slides. The slides were stained by haematoxylin and eosin. Histological indices were determined by use of a computer-aided light microscopic image analyzer (Motic Images, 2000 1.2, Scion Image, Japan). The villous height, and crypt depth were measured and calculation was made for villous height: crypt depth rate. Means values of 10 adjacent, vertically oriented villous-crypt units per section were considered for analysis.

Serum Lipids

Two birds from each replicate were randomly selected and blood samples were taken via wing vein at 42 days. Serum samples were taken and cholesterol, triglyceride, LDL and HDL were measured by using the specific kits (Pars Azmoon, Tehran) and spectrophotometer (UV) in 546 nm wavelength.

Statistical Analysis

A completely randomized design (CRD) was employed. One-way analysis of variance was performed using the general linear model procedure of SAS software [30]. Duncan's multiple range test were used as the means of comparison ($P<0.05$)

Results

Performance

The effects of treatments on performance are shown in Table 2. In the present study, FI at the age of 21 days showed significant difference between treatments ($P<0.05$). The highest and lowest feed intake was observed in negative control and Broncofin® groups, respectively. There was no significant difference in FI between treatments at 21-48 and 1-48days of age ($P>0.05$). During these periods, Bromhexin® and Antibiofin® had the highest and lowest FI among the treatments, respectively. At 21 days of age, the highest and lowest BW and BWG were observed in Immunofin® and Zagrol® treatment groups, respectively ($P<0.05$). In day48, the highest and lowest BW and BWG were observed in Bromhexin® and Zagrol® treatments, respectively ($P>0.05$). A significant difference was observed between treatments in FCR at 1-21, 22-48 and 1-48 days of age ($P<0.05$). During the whole experiment period, Immunofin® showed the lowest FCR which was significantly lower than positive control and chemical medicine groups and Zagrol® had the highest FCR ($P<0.05$). The highest and the lowest EPEF were observed in Immunofin® and positive control treatments, respectively ($P> 0.05$).

Bacterial Population in the Intestinal Contents

The effects of treatments on microflora population in ileo-cecal contents are shown in Table 3. Number of Escherichia coli in Enrofloxacin® treatment were significantly lower in comparison with other treatments except Mentofin® and negative control ($P<0.05$). The highest colony number of lactic acid bacteria belonged to Zagrol® that was significantly higher than control and Enrofoloxacin® groups ($P<0.05$) and the lowest number belonged to Mentofin® treatment. The highest colony number of total aerobic bacteria was observed in Antibiofin® treatment that was significantly higher than control groups. The lowest total aerobic bacteria were observed in the Mentofin® treatment group.

Intestinal Morphology

The effect of different treatments on intestinal morphology characteristics are presented in Table 4. Treatments affected villous characteristics. No significant differences were observed between treatments in the villous height in the duodenum, jejunum and ileum ($P>0.05$). The highest and lowest villous height in duodenum and jejunum were attained by Bromhexin® and Zagrol® respectively ($P>0.05$). Significant differences were observed between treatments in the crypt depth in duodenum, jejunum and ileum ($P<0.05$). Greatest crypt depth in duodenum, jejunum and ileum belonged to Zagrol® treatment. The highest and lowest ratio of villi height: crypt depths in jejunum and duodenum were observed in Bromhexin® and Zagrol® treatment groups.
In ileum Mentofin® had the lowest crypt depth and highest villi height: crypt depth ratio ($P<0.05$).

Serum Lipid

The effects of treatments on triglyceride, cholesterol and lipoproteins with high and low density are illustrated in Table 5. Cholesterol and LDL-cholesterol concentrations were affected by treatment ($P<0.05$), but triglyceride and HDL-cholesterol didn't show significant difference among the treatments ($P>0.05$). The lowest concentration of cholesterol, LDL, and the highest concentration of HDL was observed in Anzofin® treatment group.

Table 2 The effect of treatments on broiler performance

SEM	P-value	NC	PC	Zg	Me	Ef	Bh	Bk	Ab	Im	Az	period	Treatments
9.70	0.0001	629.78 ± 10.0^{a}	593.00 ±22.0^{ab}	497.1 ±22.9^{c}	545.33 ±11.4^{bc}	584.22 ±32.5^{ab}	588.22 ±29.4^{ab}	509.41 ±21.1^{c}	630.22 ±21.3^{a}	633.05 ±42.5^{a}	538.16 ±33.3^{bc}	1-21	BW (g)
30.91	0.43	2616.5 ± 49.2	2583.7 ±246.4	2308.2 ±171.0	2517.4 ±181.0	2545.6 ±72.4	2683.1 ±285.5	2474.4 ±138.0	2481.4 ±200.8	2552.6 ±68.5	2521.4 ±75.0	1-48	
9.67	0.0001	580.89 ± 80.7^{a}	543.22 ±23.3^{ab}	449.10 ±26.2^{c}	497.56 ±14.7^{bc}	538.00±35.4^{ab}	541.78±30.57^{ab}	463.41±21.8^{c}	580.67 ±22.7^{a}	585.05 ±45.7^{a}	492.83 ±33.1^{bc}	1-21	BWG (g)
28.97	0.74	1986.7 ± 51.3	1990.7 ±225.3	1811.1 ±155.5	1972.1 ±187.9	1961.3 ±89.1	2094.8 ±312.5	1964.9 ±119.8	1851.2 ±184.0	1919.6 ±61.4	1983.2 ±103.85	21-48	
31.05	0.44	2567.6 ± 46.9	2533.9 ±247.9	2260.2 ±175.9	2469.7 ±183.1	2499.3 ±74.2	2636.6 ±283.7	2428.4 ±139.9	2431.8 ±201.5	2504.6 ±69.6	2476.1 ±77.3	1-48	
15.37	0.0002	914.94 ± 45.5^{a}	846.46 ±60.4^{abc}	729.25 ±68.4^{cd}	778.14 ±32.1^{bcd}	863.29 ±44.4^{ab}	840.59 ±30.1^{abc}	704.88 ±26.4^{d}	889.97 ±16.7^{ab}	842.05 ±84.1^{abc}	712.99 ±56.3^{d}	1-21	FI (g)
65.39	0.57	4143.9 ±123.2	4324.6 ±461.6	4001.4 ±123.2	4157.0 ±313.0	4065.1 ±205.0	4405.0 ±712.2	4234.7 ±239.1	3773.4 ±323.7	3858.1 ±268.3	4031.1 ±255.9	21-48	
67.02	0.56	5058.8 ± 8	5171.0 ±518.8	4730.6 ±466.4	4935.2 ±310.8	4928.4 ±183.8	5245.6 ±691.9	4939.6 ±264.9	4663.4 ±328.5	4700.2 ±261.5	4744.1 ±227.3	1-48	
0.014	0.013	1.57 ± 0.06^{ab}	1.56 ±0.06^{ab}	1.62 ±0.1^{a}	1.56 ±0.03^{ab}	1.61 ±0.03^{a}	1.55 ±0.05^{ab}	1.52 ±0.04^{ab}	1.53 ±0.08^{ab}	1.44 ±0.04^{b}	1.45 ±0.03^{b}	1-21	FCR
0.013	0.0002	2.09 ± 0.03^{bc}	2.17 ±0.05^{ab}	2.21 ±0.04^{a}	2.11 ±0.04^{abc}	2.07 ±0.01^{bc}	2.10 ±0.05^{abc}	2.16 ±0.1^{ab}	2.04 ±0.05^{c}	2.01 ±0.08^{c}	2.03 ±0.3^{c}	21-48	
0.012	0.0001	1.93 ± 0.02^{bcd}	2.00 ±0.03^{ab}	2.05 ±0.05^{a}	1.96 ±0.01^{abc}	1.94 ±0.02^{bc}	1.95 ±0.05^{bc}	2.00 ±0.01^{ab}	1.88 ±0.02^{cd}	1.84 ±0.06^{d}	1.88 ±0.03^{cd}	1-48	
1.14	0.20	88.00 ± 6.9	77.67 ±6.7	91.67 ±1.5	86.34 ±7.4	85.67 ±1.5	89.67 ±8.1	89.67 ±4	84.00 ±8.5	91.00 ±1.7	89.00 ±5.2	1-48	viability
4.38	0.11	243.38 ± 25.0	203.77 ±10.4	210.89 ±14.0	227.25 ±36.7	229.86 ±4.0	250.11 ±8.3	226.83 ±17.2	227.29 ±38.6	257.64 ±7.8	243.59 ±17.4	1-48	EPEF

^{abcd} Means in columns with different superscripts were significantly different ($p<0.05$). SEM, Standard Means of Errors.

Az: Anzofin, Im: Immunofin, Ab: Antibiofin, Bk: Broncofin, Bh: Bromhexin, Ef: Enrofloxacin, Me: Mentofin, Zg: Zagrol, PC: Positive Control, NC: Negative Control.

Table 3 The effect of treatments on microbial population (Log10 cfu/g)

SEM	P-value	NC	PC	Zg	Me	Ef	Bh	Bk	Ab	Im	Az	Treatments
0.24	0.0001	4.92 ±0.04^{c}	6.25 ±0.1^{b}	7.33±0.6^{a}	4.78 ±0.05^{c}	4.41 ±0.5^{c}	5.76 ±0.4^{b}	7.49 ±0.4^{a}	8.10 ±0.5^{a}	6.52 ±2.0^{b}	7.37 ±2.0^{a}	Coliform
0.16	0.0001	6.80 ±0.1^{cd}	7.17 ±0.1^{bc}	8.13 ±0.2^{a}	5.51 ±0.15^{f}	6.17 ±0.1^{def}	6.41 ±0.1^{de}	5.71 ±0.6^{ef}	7.67 ±0.2^{ab}	7.66 ±0.6^{ab}	7.15 ±0.3^{bc}	Lactic acid bacteria
0.27	0.0004	6.17 ±0.6^{bcd}	4.70 ±0.04^{d}	7.79 ±0.04^{ab}	4.70 ±0.1^{d}	6.01 ±0.1^{bcd}	5.81 ±1.7^{bcd}	7.09 ±0.4^{abcd}	8.57 ±0.03^{a}	7.37 ±0.2^{abc}	5.21 ±2.3^{cd}	aerobic bacteria

^{abcd}Means in columns with different superscripts were significantly different ($p<0.05$). SEM, Standard Means of Errors.

Az: Anzofin, Im: Immunofin, Ab: Antibiofin, Bk: Broncofin, Bh: Bromhexin, Ef: Enrofloxacin, Me: Mentofin, Zg: Zagrol, PC: Positive Control, NC: Negative Control.

Table 4 The Intestine histomorphological parameters of broilers at 48 days age

Treatments Intestine morphology	Az	Im	Ab	Bk	Bh	Ef	Me	Zg	Pc	Nc	P-value	SEM
Villi height (µm)												
Duodenum	1279.40 ±75.6	1308.49 ±64.7	1293.12 ±156.0	1278.91 ±49.9	1321.45 ±45.3	1287.01 ±75.1	1286.40 ±19.9	1262.17 ±10.1	1301.85 ±18.4	1267.14 ±145.4	0.99	12.77
Jejunum	1123.72 ±42.8	1080.90 ±45.6	1099.67 ±77.8	1087.10 ±41.9	1128.42 ±52.8	1047.94 ±72.9	1115.18 ±11.9	1040.90 ±50.8	1109.71 ±12.7	1068.14 ±66.5	0.44	9.57
Ileum	861.99 ±15.9	907.81 ±20.2	881.53 ±18.1	893.62 ±22.7	857.39 ±5.1	857.98 ±33.1	899.02 ±55.5	868.42 ±23.8	888.18 ±87.7	855.76 ±24.0	0.72	6.60
Crypt depth (µm)												
Duodenum	73.17 ±13.7^{b}	77.65 ±28.0^{b}	155.5 ±45.8^{ab}	96.42 ±57.8^{b}	73.22 ±56.0^{b}	106.42 ±25.7^{b}	87.94 ±6.6^{b}	234.44 ±88.9^{a}	148.67 ±11.5^{ab}	104.70 ±9.5^{b}	0.0012	10.75
Jejunum	86.70 ±6.4^{c}	106.80 ±17.53^{bc}	132.48 ±17.6^{bc}	157.74 ±58.180^{b}	80.97 ±6.7^{c}	96.47 ±29.6^{c}	132.37 ±57.5^{bc}	220.74 ±8.1^{a}	118.52 ±7.7^{bc}	103.98 ±13.4^{bc}	0.0004	8.53
Ileum	68.05 ±7.2^{c}	78.54 ±10.5^{bc}	188.68 ±72^{ab}	125.98 ±50.8^{abc}	128.32 ±35.6^{abc}	76.24 ±20.5^{bc}	65.09 ±7.0^{c}	219.11 ±30.6^{a}	188.74 ±70.7^{ab}	148.35 ±65.6^{abc}	0.0017	12.05
Villi height: Crypt depth												
Duodenum	17.82 ±2.7^{a}	18.36 ±6.3^{a}	8.70 ±2.0^{ab}	16.23 ±7.7^{a}	18.13 ±1.7^{a}	12.68 ±3.6^{ab}	15.20 ±2.0^{a}	5.90 ±2.0^{b}	8.80 ±0.7^{ab}	12.14 ±1.6^{ab}	0.002	0.96
Jejunum	13.02 ±1.3^{ab}	10.35 ±2.1^{abc}	8.45 ±1.7^{bcd}	7.46 ±2.3^{cd}	14.01 ±1.2^{a}	11.82 ±4.8^{abc}	9.90 ±5.2^{abc}	4.71 ±1^{d}	9.38 ±0.6^{abcd}	10.34 ±0.6^{abc}	0.012	0.62
Ileum	12.76 ±1.3^{ab}	11.72 ±1.8^{ab}	5.08 ±1.6^{c}	8.08 ±3.7^{abc}	7.15 ±1.8^{bc}	11.83 ±3.2^{ab}	13.88 ±1.3^{a}	4.01 ±0.5^{c}	5.38 ±2.7^{c}	6.82 ±3.6^{bc}	0.0002	0.73

^{abcd}Means in columns with different superscripts were significantly different ($p<0.05$). SEM, Standard Means of Errors.

Az: Anzofin, Im: Immunofin, Ab: Antibiofin, Bk: Broncofin, Bh: Bromhexin, Ef: Enrofloxacin, Me: Mentofin, Zg: Zagrol, PC: Positive Control, NC: Negative Control.

Table 5 The effect of treatments on serum lipids of broilers (Mg/dl)

Treatments	Az	Im	Ab	Bk	Bh	Ef	Me	Zg	PC	NC	P-value	SEM
CH[1]	148.48 ±1^{b}	157.58 ±25.5^{b}	248.15 ±42.8^{a}	160.61 ±58.8^{ab}	182.49 ±13.4^{ab}	205.72 ±52.3^{ab}	248.24 ±12.7^{a}	231.31 ±45^{ab}	222.80 ±22.8^{ab}	166.33±11.1^{ab}	0.005	8.57
TG[2]	79.10 ±12.1	103.90 ±91.8	69.57 ±22.8	114.70 ±58.3	55.49 ±18.0	107.70 ±8.7	55.00 ±17.6	74.90 ±28.9	60.00 ±8.45	82.80 ±18.8	0/47	6.94
HDL[3]	53.04 ±16.9	47.22 ±13.2	44.70 ±5.8	44.29 ±12.6	45.31 ±3.8	39.22 ±10.2	40.11 ±10.2	45.38 ±11.4	47.09 ±11.1	37.65 ±8.2	0.85	1.83
LDL[4]	79.62 ±18.3^{d}	89.56 ±18.8^{cd}	189.54 ±37.3^{abcd}	93.38 ±45.6^{bcd}	126.08 ±15.1^{d}	144.97 ±63.7^{abcd}	197.12 ±21.0^{a}	170.94 ±33.7^{ab}	163.71 ±12.0^{abc}	112.12 ±16.8^{bcd}	0.001	8.99

^{abcd}Means in columns with different superscripts were significantly different ($p<0.05$). SEM, Standard Means of Errors.

[1]CH=Cholesterol; [2]TG=Triglyceride; [3]HDL= High density lipoprotein; [4]LDL= Low density lipoprotein.

Az:Anzofin, Im: Immunofin, Ab: Antibiofin, Bk: Broncofin, Bh: Bromhexin, Ef: Enrofloxacin, Me: Mentofin, Zg: Zagrol, PC: Positive Control, NC: Negative Control.

Discussion

The majority of experimental results indicates reduced feed intake at largely unchanged body weight gain or final body weight, leading to an improved feed conversion ratio when feeding essential oils [2]. Other studies have also investigated the effects of phytobiotic supplements on different poultry species. On average, phytobiotics containing plant extracts could improve FCR by decreasing the feed intake, without developing significant changes in body weight, and daily body weight gain, and the results of the current study confirmed these findings.

On the 21 days of the experiment, Anzofin® and Broncofin® treatments reduced FI and had a desirable impact on performance. Lowest FCR was observed in Anzofin®, Immunofin® and Broncofin® treatments, respectively. Throughout the experiment, Antibiofin® and Immunofin® had a positive effect on FCR by reducing FI. Bromhexin® treatment in 1- 48 days of age had the highest FI, BW and BWG, but its effect on FCR was not significant. Bromhexin® is a chemical bronchodilator with expectorant properties. This drug, increases secretion of mucus, diluting sputum and helping it exit from trachea and opening the trachea, that makes breathing easier and can cause faster recovery of respiratory diseases. Therefore, it could increase FI and BWG through improving the chicken health conditions. The EOSk (Zagrol®) consists of a wide spectrum of volatile lipophilic compounds including carvacrol which comprises more than 92 percent of the whole extract. Carvacrol is a bitter-tasted, pungent agent which obviously changes the flavor of water and causes a significant reduction of water consumption in broilers. So, observation of the low BWG and BW, decrease in FI and increased FCR in Zagrol® treatment throughout this experiment, is perhaps due to the high percentage of carvacrol in combination of this commercial medicine. As mentioned above, Immunofin is an extract of Purple coneflower (*Echinacea purpurea*). Purple coneflower belongs to the group of phytogenic compounds that helps to create and strengthen immune system network through improving the immune system [31,32], so it can improve the health status of animal. In Europe, Echinacea is known as a medication improving immune system. Purple Coneflower contains various active ingredients such as alkamides, glycoproteins, polysaccharides, phenolic compounds, cinamic acids, essential oils, and flavonoids that are effective in the treatment of many diseases [33,34]. It has been reported that the immune stimulation may have adverse effects on animal growth, because the nutrients are distributed and used mostly for antibody synthesis and the growth of the immune organs. Therefore, the available nutrients which are required for animal growth will decrease [35]. So, Purple coneflower with immunomodulator properties [36] could have some impacts on the growth. Klasing [37] reported that immune stimulation cannot generally reduce the growth. Immune system nutrient requirements are much less than required nutrients for growth. However, the effects of immune stimulation differ from the immune stresses that cause infectious disease. As mentioned before, in this experiment the treatments were challenged with infectious bronchitis disease vaccine. This challenge can influence the health status and prevent the optimum growth of the chickens in the framework of the genetic potential. However, given the growth rate, FCR, percentage of livability and EPEF in Immunofin® treatment group, the researcher comes to conclusion that this herbal medicine improves the performance and health status of the birds. Such impact indicates that phytogenic compound and their active ingredients have more diverse activities in the animal body, having effects on different physiological pathways and on the immune system. Since the EPEF shows the amount of feed efficiency as well as the percentage of the losses in addition to the body weight, therefore, it is a good criterion for the best use of the ration. On the other hand, based on this index, if there were any losses due to the administration of experimental treatments, they will show their impact on the result of the experiment. So the amounts of viability and FCR in Immunofin® treatment group justify the highest EPEF compared to other treatments. Zagrol® treatment, in spite of having the highest percentage of viability compared to other groups, which is due to the adverse effect on FCR, in terms of EPEF, was in the lowest after positive control group.

Savory is full of various vitamins, especially vitamin A and E. It is possible that these vitamins play an important role in the production of antibody, and increasing the level of serum antibody and phagocytic activity of immune system cells in chickens [38,39]. Therefore, this could be the reason of improvement of health status and viability percentage in this treatment. But, avoiding using any medication and growth promoter in the PC group caused the increase of FCR and reduced the health status and livability and thus reduced EPEF.

The intestinal microbiota plays a vital role in the normal nutritional, physiological, immunological, and protective functions of the host animals [40]. The

composition and metabolic activity of the intestinal microbiota can be influenced by the diet [41]. It is suspected that addition of antibiotics or essential oils may be efficient at reducing the pathogen load [42]. So with respect to animal production, an important goal is to achieve the optimal microflora for the animal (maximum benefits with minimum costs) and manipulate the microflora through diet, supplements, etc. to obtain the desired microflora.

Enrofloxacin® medicine was significantly more successful in reducing the overall coliform bacteria compared to other treatments. Jang et al. [43] reported that number of *E. coli* in ileocecal contents in groups that had consumed antibiotics was significantly lower than the control group. The operational mechanism of antibiotic, as a growth promoter is in relationship via its interaction with the microbial population of GI [44] and this compound can improve the growth and FCR through moderating or omitting the harmful microorganism that exist in GI. Enrofloxacin® and Mentofin® treatments had the lowest number of coliform bacteria. Jang et al. [43] observed there was a similar CFU number of E. coli among birds fed the basal diets fortified with antibiotics and two levels of essential oil. The lowest number of aerobic bacteria and *Lactobacillus* was also observed in Mentofin® treatment. Mentofin® is a commercial herbal medicine contained in the essential oils of peppermint and *Eucalyptus* [24]. It has been reported that Peppermint Oil [45-47] and the extract of Eucalyptus leaf have antimicrobial effects [48]. Phytochemical compounds show antibacterial activities through the various mechanisms. For example, essential oils can damage the cell wall and membrane leading to the leakage of macromolecules and lysis of bacteria [49,50]. This is due to the lipophilic property of essential oils that make them pass through the cell wall and cytoplasmic membrane, and damage the cell [23]. It was expected that reducing the number of total aerobic and coliform bacteria cause the increase of lactic acid bacteria colonization in the Mentofin® treatment group, but number of lactic acid bacteria in this treatment was decreased. Mentofin® didn't have a differential inhibition between beneficial intestinal microflora and harmful Enterobacteria and so decreased all of the three colonies. Differential inhibition between beneficial and harmful intestinal microflora may be due to differing composition of bacterial membranes and their permeability to medicine components which are consumed [51].

The benefit of lactic acid bacteria seems to have stemmed from its production of bacteriocins, and the benefit appears to be associated with the production of bacteriocins of some species which help competitive exclusion of harmful and pathogenic microorganisms (such as *Salmonella*, Enterococci, and *Escherichia*). As it is mentioned, carvacrol is formed more than 92 percent of the whole extract of Zagrol® medicine. It has a stimulating effect on Lactobacillus proliferation [52]. So carvacrol can be the cause of the observation of highest number of lactic acid bacteria in the Zagrol® treatment. It is well known that many substances can affect the intestinal villi development [27]. But, there is only slight evidence of morphological and histological investigations referring to active plant oils action in animals fed on diets supplemented with plant extracts. Both villous height and crypt depth are important indicators of broilers digestive health and directly related to the absorptive capacity of mucous membrane [53]. The highest and lowest villous height and villous: crypt ratio in the duodenum and jejunum were attained by Bromhexin® and Zagrol® treatment groups respectively. From a theoretical point of view, villous height reflects a balance between the mitotic activity of the crypt enteric cells [54] and the desquamation produced principally by external aggressors [55]. Also the villous: crypt ratio is an indicator of the likely digestive capacity of the small intestine. An increase in this ratio corresponds to an increase in digestion and absorption [56]. On the other hand, a decrease in villus: crypt ratio is indicative of a higher rate of enterocyte-cell migration from the crypt to the villous. However, Bromhexin® despite the positive effect on morphology couldn't effect on performance. Intestinal cell proliferation occurs mainly in the crypts [29]. Thus, the large crypt suggests a high nutrient requirement for intestinal maintenance and reduced efficiency of the bird. In this view, the large crypt and small villous: crypt ratio birds which consumed Zagrol® may partly explain the poor growth performance.

The lowest amount of cholesterol, LDL, and the highest HDL concentration were observed in the Anzofin® treatment group that confirms positive effects of herbal medicine on serum lipids. Some of the active compounds of plants can inhibit the activity of the number of lipogenenic enzymes including hydroxymetylglutaryl (HMG) CoA reductase, acyle CoA: cholesterol acyltransferase (ACAT), microsomal triglyceridetransferase (DGAT) [52,57,58]. Also medicinal plants with immunostimulatory effect use the available energy and prevent the build-up of cholesterol and fat [59]. Herbal can lead to increase digestive secretions such as bile acids [60]. Cholesterol is broken by bile acids

and therefore not made again. There is also a belief that the more secretion of bile acids from the intestinal cavity under the influence of herbal ingredients will make the cholesterol spent to build bile acids and ultimately reduce serum cholesterol [61].

Conclusion

The chickens in this research were examined with the Infectious Bronchitis vaccine virus so a proportion of their energy was expended to overcome this challenge. In this condition, Immunofin®, Antibiofin® and Anzofin® were more effective in improving the growth performance of the birds compared to chemical medicine and control groups. The results of this experiment recommend the use of these compounds as growth promoter and antibiotics alternatives in poultry feed. This challenge caused the experimental condition becoming closer to commercial farm conditions and reviewing the effects of these medicines on the health status and performance of birds in such conditions.

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