The Effects of Comparison of Herbal Extracts, Antibiotic, Probiotic and Organic Acid on Serum Lipids, Immune Response, GIT Microbial Population, Intestinal Morphology and Performance of Broilers

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Receive: 19 Jan 2011 **Acceptance:** 12 Feb. 2011

Abstract

Background: With the removal of antibiotic as growth promoters from poultry diets, it is of interest to investigate potential alternatives to maintain good growth performance and good intestinal microbial populations in these birds. Numerous additives such as Probiotics, prebiotics, organic acids, enzymes and herbal extracts used extensively in poultry feed.

Objective: The study was conducted to investigate the effects of herbal extracts, probiotic, organic acid and antibiotic on serum lipids, immune response, intestinal morphology, microbial population and performance of broilers.

Method: A total of 300 day - old male broilers (Cobb 500) were randomly divided into four treatments, five replicates with 15 birds in each. Treatments included: control, herbal extracts (Sangrovit®), probiotic (Primalac®), organic acid (Termin-8®) and antibiotic (Virginiamycin).

Results: The highest weight gain (WG) were achieved by virginiamycin ($p<0.05$). Moreover, highest and lowest antibody titers against SRBC were observed in Primalac® and virginiamycin treatments, respectively ($p<0.05$). Lowest serum cholesterol and triglyceride were obtained by Primalac® and Sangrovit® ($p<0.05$). The lowest and highest coliform bacteria counts in ileum were seen in virginiamycin and control groups, respectively ($p<0.05$).

Conclusion: It has been concluded that Sangrovit®, Primalac® and Termin-8® reduced pathogenic bacteria in digestive tract of broilers, which can help to improve intestinal health of these animals. Thus, these can be used as antibiotic alternatives in broilers feed.

Keywords: Virginiamycin, Primalac®, Termin - 8®, Sangrovit®, Broiler performance

[DOR: 20.1001.1.2717204.2011.10.37.10.1]

Introduction

Antibiotics have been used for more than half a century in poultry feed for improving performance, reducing some pathogenic microorganisms and increasing some useful microorganisms in intestinal tract of these birds [23]. However, antibiotics used as growth promoters in animal feeds have been banned recently due to potential development of antibiotic resistant human pathogenic bacteria [49]. With the removal of antibiotic growth promoters from poultry diets in different areas of the world, it is of interest to investigate potential alternatives to maintain good growth performance and good intestinal microbial populations, particularly to control the growth of harmful bacteria. Numerous additives are used or proposed as means to reduce or eliminate pathogens or improve growth and FCR [32]. Probiotics [2], prebiotics [6], organic acids [27], enzymes [70] and herbal extracts [55] used extensively in poultry feed.

Probiotics are organisms and substances which help to improve the environment of the intestinal tract. Probiotics may be defined as living microorganism which given to animals, assist in the establishment of an intestinal population which is beneficial to the animal and antagonistic to harmful microbes [25]. Also, important characteristics of probiotics are improving feed efficiency without any residual in the poultry tissue and resulting in diseases resistance [62]. In addition, probiotics not only are used as a growth promoter, but also induce immune system and have protective effects against many diseases [23].

Organic acids are the candidate alternative for antibiotics, either individual acids or blends of several acids [8]. Several organic acids have been reported to improve growth performance, feed efficiency, mineral absorption, and phytate-P utilization when supplemented in diets [5]. Acidification with various organic acids has been reported to reduce the production of toxic components by the bacteria and colonization of pathogens on the intestinal wall, thus preventing the damage to epithelial cells. Langhout [38], also improve the digestibility of proteins, calcium, phosphorus, magnesium, and zinc that serve as substrates in the intermediary metabolism.

Another antibiotic alternative are herbal extracts, which are being used as feed supplements to improve growth performance, manipulation of gut functions and microbial habitat of domestic animals [48]. Herbal extracts supplements have shown to have beneficial effects on broiler performance and carcass quality [60]. A variety of herbal supplements have been widely used to maintain and improve health of humans [19] and birds [22]. Herbal extracts can also improve immune system [42] and reduce blood cholesterol [55]. Sangrovit® is a phytogenic feed additive that is extracted from some plants including *Sanguinaria canadensis*. Sanguinarine is a quaternary ammonium salt from the group of benzylisoquinoline alkaloids. It has been shown that sanguinarine suppresses the growth of some bacteria that cause gastrointestinal distress [41], enhance appetite and feed intake, and promote growth [66]. There are many inconsistent results regarding substitution of probiotic, organic acids and herbal extracts for antibiotic and clarifying roles of these additives in poultry production. The prevention of diseases and enhancement of growth, FI and feed efficiency are critical factors in modern broiler production. Therefore, the objective of present study was to evaluate and compare the effects of herbal extracts (Sangrovit®), probiotic (Primalac®),

[DOR: 20.1001.1.2717204.2011.10.37.10.1]

organic acid (Termin-8®) and antibiotic (Virginiamycin) on serum lipids, immune response, intestinal morphology, microbial population and performance of broilers.

Materials and Methods

Experimental design and husbandry

A total of 300 one-day old male broilers (Cobb 500) were randomly allocated to 4 treatments, 5 replicates with 15 birds in each. Dietary treatments were basal diet (control), virginiamycin (Phibro, U.S.A; 15 ppm of diet), Primalac® (Star–Labs, U.S.A; 0.1 % of diet), Termin-8® (Anitox, U.S.A; 0.2 % of diet) and Sangrovit® (Phytobiotics, Germany; 35g/ton of diet) which these additives were administrated for a 42 days period. Feed and water were provided *ad libitum* throughout the study. Lighting schedule was 23L/1D. The temperature was gradually reduced from 32°C by increments of 3°C in each week. Feed composition and formulation of starter (1-14 days), grower (15-28 days) and finisher (29-42 days) diets were based on NRC [47] which presented in Table 1**.** Feed intake (FI), BWG and FCR were measured weekly throughout the experiment period.

Immunity and serum lipid assay

Two birds from each replicate were injected with 1 mL 5% sheep red blood cells (SRBC) antigen suspended in 0.9% saline for evaluation of immune system responses at 21 and 35 days. Blood samples were taken via wing vein at 28 and 42 days and stored at -20°C. The agglutination assays for SRBC antigen were carried out as described by Munns and Lamont [46]. Sera were analyzed for total titers, and IgG and IgM. The Mercaptoethanol- resistant (MER) titers and mercaptoethanol-sensitive (MES) titers were determined. The MER and MES titers in broilers are presumably, the same as other species [50].

Table 1- Diet formulation and calculated chemical composition

Ingredients (%)	Starter (1 - 14)	Grower (15 - 28)	Finisher (29 - 42)
Corn	49.82	52.11	47.09
Soybean meal	41.08	35.03	30.96
Wheat	4.2	8.09	14.68
Soybean oil	1.1	1.29	4.23
Dicalcium phosphate	2.46	2.20	2.06
DL-methionine	0.34	0.26	0.16
L-lysine	0.23	0.19	0.03
Vitamin permix[1]	0.25	0.25	0.25
Mineral permix[2]	0.25	0.25	0.25
Limestone	-	0.05	-
Salt	0.27	0.28	0.28
Calculated analysis			
ME (kcal/kg)	2820	2950	3045
Crude protein%	21.53	18.85	18.01
Crude fat%	4.04	5.05	6.57
Calcium%	0.93	0.83	0.80
Available P%	0.47	0.41	0.40
Methionine + cystine %	0.9	0.82	0.72

1- Supplied the following per kilogram of diet: vitamin A (retinyl acetate), 8,000 IU; vitamin D_3 (cholecalciferol), 3,000 IU; vitamin E (DL-alpha-tocopheryl acetate), 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 (pyridoxine_HCl), 4 mg; riboflavin, 10 mg; and thiamin, 3 mg (thiamin mononitrate).

2- Supplied the following per kilogram of diet: 10 mg of copper (CuSO4); 1.0 mg of iodine Ca (IO3) 2; 80 mg of iron (FeSO4_H2O); 100 mg of manganese (MnSO4_H2O); 0.15 mg of selenium (NaSeO3); 80 mg of zinc (ZnSO4_H2O); and 0.5 mg of cobalt (CoSO4).

[DOR: 20.1001.1.2717204.2011.10.37.10.1]

Broilers 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, 2009) and spectrophotometer (UV) in 546 nm wavelength.

Microbial sampling and incubation

On day 42 of experiment, two birds from each replicate were killed via CO_2 inhalation. The crop, ileum and cecum contents were collected. Contents were gently removed in sterile sampling tubes and immediately transferred on ice to the laboratory. The contents of mentioned segments were used for microbial study. The serial dilutions (10^{-3} to 10^{-7}) were made. Thereafter, the selective media of Plate Count Agar (Merck, Germany), De Man Rogosa Sharpe Agar (MRS) (Merck, Germany) and MacConkey Agar (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 hours and *lactic acid* bacteria after aerobic incubation at 37°C for 48 hours [72].

Intestinal morphology assay

On 21 and 42 days of age middle sections (3-4 cm) of duodenum, jejunum and illume of two birds from each replicate were cut and prepared for histological indices assay. The histological indices were measured according to Iji et al. [31] method. Intestinal tissue samples were fixed in formalin and dehydrated, cleared, impregnation with paraffin. The processed tissue was then embedded in paraffin wax. Section were cut (6µm) from the waxed tissue on LEICA RM 2145 microtome, cleared of wrinkles by floating on warm water (55- 60°C) 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, 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.

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 [59]. Duncan's multiple range test were used to means comparison ($p<0.05$).

Results

Performance

The effects of dietary treatments on broiler performance are presented at Table 2. No significant differences were found between treatments in FI at 1 - 14, 15 - 28, 29 - 42 and 1 - 42 days of age ($p>0.05$). Moreover, no significant differences were obtained in WG at 1 - 14 and 14-28 days of age ($p>0.05$). The highest and lowest WG was achieved in virginiamycin and control at 28-42 and 1 - 42 days of ages, respectively ($p<0.05$). Also, no significant differences were observed in FCR between treatments at 1 - 14 and 14-28 days of age ($p>0.05$). The highest FCR were obtained by control at 28 - 42 and 1 - 42 days of age ($p<0.05$).

Immunity assay

The results of dietary treatments on immune response of broilers are displayed in Table 3. The primary immune response against

[DOR: 20.1001.1.2717204.2011.10.37.10.1]

SRBC was not affected by the treatments ($p>0.05$), but secondary immune response and heterophil to lymphocyte ratio (H/L) were significantly affected ($p<0.05$). The highest and lowest total anti-body titers against SRBC and IgG and IgM were observed in Primalac® and virginiamycin, respectively ($p<0.05$). Moreover, Primalac® and Sangrovit® treatments caused significant increases in relation H/L rather than other treatments ($p<0.05$).

Table 2 - Broilers performance in response to different treatments

Measurement	Treatments					SEM
	Control	Primalac®	Sangrovit®	Virginiamycin	Termin-8®	
Feed intake (g/day)						
1-14 day	39.53	39.31	39.01	39.10	39.70	0.344
14-28 day	105.06	106.39	106.20	106.29	106.82	0.577
28-42 day	167.33	174.67	164.33	171.44	167.33	2.15
1-42 day	105.89	107.37	105.08	104.8	106.41	0.576
Weight gain (g/day)						
1-14 day	28.39	29.31	27.71	30.11	29.06	0.393
14-28 day	48.36	55.16	57.27	56.54	53.52	1.43
28-42 day	70.89^{b}	76.55^{ab}	71.93^{b}	81.47^{a}	70.92^{b}	1.51
1-42 day	50.27^{b}	54.76^{ab}	53.36^{ab}	57.5^{a}	50.89^{b}	0.923
Feed conversion ratio						
1-14 day	1.39	1.34	1.41	1.29	1.36	0.016
14-28 day	2.19	1.92	1.87	1.88	2.00	0.049
28-42 day	2.36^{a}	2.28^{ab}	2.28^{ab}	2.10^{b}	2.34^{a}	0.036
1-42 day	2.11^{a}	1.96^{ab}	1.97^{ab}	1.82^{b}	2.09^{a}	0.034

^{abc} Means in rows with different superscripts were significantly differ ($p<0.05$). SEM: Standard Means of Errors

Table 3 - The effects of dietary treatments on immune response of broilers

Treatment	Post first immunization				Post second immunization			
	Total titer	IgG	IgM	H/L	Total titer	IgG	IgM	H/L
Control	3.91	2.78	1.20	23.87	4.57^{cb}	3.45^{ab}	1.12^{ab}	23.41^{c}
Primalac®	4.08	2.75	1.33	26.75	5.66^{a}	4.02^{a}	1.63^{a}	29.97^{a}
Sangrovit®	4.12	3.24	0.88	25.76	5.36^{ab}	3.81^{a}	1.54^{ab}	29.69^{a}
Virginiamycin	3.97	2.78	1.20	24.86	4.36^{c}	3.30^{b}	1.06^{b}	24.73^{cb}
Termin-8®	4.01	2.83	1.18	25.44	4.92^{abc}	3.61^{a}	1.31^{ab}	26.35^{b}
SEM	0.223	0.227	0.083	0.546	0.160	0.094	0.119	0.748

^{abc}Means in columns with different superscripts were significantly differ ($p<0.05$). SEM, Standard Means of Errors
H/L: hetrophil for lymphocytes

[DOR: 20.1001.1.2717204.2011.10.37.10.1]

Serum lipid assay

The effects of treatments on triglyceride, cholesterol, high and low density lipoproteins are illustrated in Table 4. The highest and lowest serum cholesterol and triglyceride levels were attained by virginiamycin and Sangrovit® groups, respectively ($p<0.05$). In addition, the highest and lowest high density lipoprotein (HDL) and low density lipoprotein (LDL) levels were respectively obtained by virginiamaycin and Primalac® ($p<0.05$).

Microbial populations

The effects of treatments on microflora population of crop, ileum and cecum are shown at Table 5. No significant differences were observed in total aerobic bacteria in ileum and cecum at 21 day of age ($p>0.05$). The lowest and highest coliforms bacteria counts in ileum at 21 days of age were achieved in virginiamycin and control, respectively ($p<0.05$). No significant differences were observed in coliforms bacteria counts in cecum at 21 day of age ($p>0.05$). Moreover, the highest and lowest *lactic acid* bacteria in crop, ileum and cecum at 21 days of age were observed in Primalac® and virginiamycin, respectively ($p<0.05$). No significant differences were observed in total aerobic bacteria in ileum at 42 day of age ($p>0.05$). Additionally, the lowest coliforms bacteria counts in ileum and cecum were attained by virginiamycin ($p<0.05$) which did not differ with Primalac® at 42 days of age ($p>0.05$). The highest lactic acid bacteria in ileum were achieved by Primalac® ($p>0.05$). Moreover, No significant differences were observed in coliforms bacteria counts and *lactic acid* bacteria in cecum at 42 day of age ($p>0.05$).

Intestinal morphology

The effect of different treatments on intestinal morphology characteristics are presented at Table 6. Dietary treatment affected villous characteristics at different age. The highest and lowest villous height in duodenum and jejunum were attained by Primalac® and control at 21 and 42 days of age, respectively ($p<0.05$). Moreover, No significant differences were observed between treatments in the villous height in ileum at 21 and 42 days of age ($p>0.05$). In addition, no significant differences were observed between treatments in the crypt depth in duodenum, jejunum and ileum at 21 and 42 days of age ($p>0.05$). Greatest villi height: crypt depths in jejunum were obtained by Primalac® at 21 days of age ($p<0.05$). Furthermore, No significant differences were observed between treatments in the villi height: crypt depth in duodenum and ileum ($p>0.05$). Greatest villi height: crypt depth in Duodenum and jejunum were obtained by Primalac® ($p<0.05$) which was not differ with Sangrovit® and Termin-8® at 42 days of age ($p>0.05$).

Table 4 - The effects of treatments on some blood parameters of broilers (Mg/dl)

Treatments	TG[1]	CH[2]	HDL[3]	LDL[4]
Control	49.86^{ab}	137.72^{ab}	54.17^{ab}	61.52^{b}
Primalac®	47.06^{b}	125.50^{b}	52.09^{b}	57.09^{c}
Sangrovit®	46.97^{b}	124.43^{b}	52.72^{b}	58.32^{bc}
Virginiamycin	52.67^{a}	140.87^{a}	59.16^{a}	65.32^{a}
Termin-8®	47.36^{b}	130.19^{ab}	53.15^{ab}	59.19^{bc}
SEM	0.7	2.43	0.980	0.889

^{abc}Means in columns with different superscripts were significantly differ ($p<0.05$). SEM, Standard Means of Errors [1]TG=Triglyceride; [2]CH=Cholesterol; [3]HDL= High density lipoprotein; [4]LDL= Low density lipoprotein

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Table 5 - The effects of dietary treatments on crop and intestine microbial population of broilers (Log_{10} cfu/g)

Measurement	Dietary treatment					SEM
	Control	Primalac®	Sangrovit®	Virginiamycin	Termin-8®	
Crop day 21						
Total aerobic	8.17	8.15	8.13	8.06	8.10	0.023
Lactic acid bacteria	7.32^{ab}	7.38^{a}	7.36^{ab}	7.29^{b}	7.31^{ab}	0.011
Total coliforms	3.37	3.37	3.35	3.30	3.32	0.015
Crop day 42						
Total aerobic	9.11^{a}	8.99^{ab}	8.97^{ab}	8.84^{b}	8.94^{b}	0.028
Lactic acid bacteria	8.06	8.20	8.12	8.13	8.11	0.019
Total coliforms	4.21	4.19	4.18	4.13	4.14	0.014
Ileum day 21						
Total aerobic	8.23	8.19	8.16	8.13	8.16	0.016
Lactic acid bacteria	8.02^{b}	8.15^{a}	8.13^{a}	8.00^{b}	8.09^{ab}	0.018
Total coliforms	5.52^{a}	5.42^{b}	5.47^{ab}	5.30^{c}	5.35^{c}	0.020
Ileum day 42						
Total aerobic	9.11	9.05	9.04	9.00	9.03	0.017
Lactic acid bacteria	8.94^{b}	9.22^{a}	9.11^{ab}	8.86^{c}	9.03^{bc}	0.032
Total coliforms	6.49^{a}	6.13^{cb}	6.42^{a}	6.04^{c}	6.20^{b}	0.042
Cecum day 21						
Total aerobic	7.94	7.88	7.93	7.83	7.85	0.019
Lactic acid bacteria	9.34^{b}	9.51^{a}	9.46^{a}	9.30^{b}	9.42^{ab}	0.021
Total coliforms	8.05	7.98	8.03	7.93	7.95	0.023
Cecum day 42						
Total aerobic	9.14^{a}	8.75^{cb}	8.79^{b}	8.67^{c}	8.72^{cb}	0.040
Lactic acid bacteria	10.13	10.30	10.26	10.10	10.21	0.032
Total coliforms	8.90^{a}	8.82^{cb}	8.87^{a}	8.77^{c}	8.80^{b}	0.020

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

Table 6 - The Intestine histomorphological parameters of broiler at 42 days age

Intestine morphology	Treatments					SEM
	Control	Primalac®	Sangrovit®	Virginiamycin	Termin-8®	
Villi height (µm 21day)						
Duodenum	934.13^{b}	1020^{a}	995.09^{ab}	942.89^{b}	1009^{ab}	12.27
Jejunum	732.84^{b}	835.01^{a}	778.29^{ab}	737.34^{b}	797.89^{ab}	13.09
Ileum	422.89	508.67	484.17	435.15	502.71	14.27
Crypt depth (µm 21day)						
Duodenum	190.65	194.12	184.27	182.60	192.67	8.01
Jejunum	155.69	132.24	142.88	148.21	141.76	5.35
Ileum	116.09	101.92	112.17	113.06	105.47	4.43
Villi height:Crypt depth 21day						
Duodenum	5.21	5.48	5.56	5.27	5.46	0.275
Jejunum	4.75^{b}	6.66^{a}	5.55^{ab}	5.01^{ab}	5.77^{ab}	0.270
Ileum	3.78	5.00	4.42	4.01	4.84	0.211
Villi height (µm 42day)						
Duodenum	1204^{b}	1264^{a}	1237^{ab}	1207^{b}	1236^{ab}	6.79
Jejunum	967.34^{b}	1088^{a}	1036^{ab}	982.09^{b}	1055^{ab}	15.33
Ileum	712.89	798.68	774.17	725.15	792.71	14.27
Crypt depth (µm 42day)						
Duodenum	228.44	224.92	222.67	226.68	224.97	1.59
Jejunum	207.42	180.20	183.42	205.17	182.17	4.69
Ileum	159.79	139.17	155.69	155.17	145.40	4.11

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Continue Table 6 -The Intestine histomorphological parameters of broiler at 42 days age

Intestine morphology	Treatments					SEM
	Control	Primalac®	Sangrovit®	Virginiamycin	Termin-8®	
Villi height: Crypt depth 42day						
Duodenum	5.28[b]	5.62[a]	5.55[ab]	5.33[b]	5.50[ab]	0.046
Jejunum	4.66[b]	6.28[a]	5.64[ab]	4.79[b]	5.81[ab]	0.202
Ileum	4.58	5.73	5.05	4.74	5.48	0.184

[abc] Means in rows with different superscripts were significantly differ (P<0.05). SEM: Standard Means of Errors

Discussion

Performance

Controlling the growth of intestinal microflora is important for improving the well-being of the host. Good intestinal health will lead to a better growth rate and feed efficiency in poultry [44]. It is known that antibiotics reduce pathogenic bacteria [43] in intestine. This action causes a reduction of competition for microbial nutrients in the host and thereby increases availability of nutrients [71]. This can improve FCR and increase BWG [3, 67, 16]. On the other hand, Miles et al. [43] has shown that antibiotics result in a thinner muscularis mucosa. This could lead to an increased absorption efficiency of nutrients and lead to a better performance [39]. Bafundo et al. [3] Ferket, [18] and Cabuk et al. [7] have reviewed the various benefits of antibiotics in broiler diets. Results of current study are in agreement with findings of these researches. Antibiotics induce enlargement of villous length and width along with a decrease of depth of intestinal crypts, which can be correlated with a decrease of total microbial population.

The improvement in performance and feed efficiency of broiler chickens fed probiotics [34, 45] is known to be via retention of beneficial microbial population in digestive tract and improving feed digestion and absorption [20]. Furthermore, probiotics increase short-chain fatty acids [56], decrease intestinal pH [29], improve intestinal morphology and immune response [35]. The positive effects of probiotics on broiler performance in this experiment are in agreement with studies of some researchers [34, 35]. The recent scientific article refers to dietary supplementation with extracts of some plants indicating growth promotion, nutrient digestibility enhancement, and feed efficacy mechanisms in broiler chickens [7, 21]. Results of studies by Tschirner et al. [66] and Vieira et al. [69] indicated benefit effects of using Sangrovit® in broilers and swine diets. Moreover, Sangrovit® significantly increased SCFA's in intestine, improves gut characteristics and nutrient absorption [33]. These data support the benefits of Sangrovit® on WG and FCR observed in our finding. It has been suggested that organic acids improve the microflora and reduce harmful bacteria of the gut [53] and with producing an appropriate pH in the gut [63] and on the other hand increasing the metabolism energy with the use of enzymes [64], they can increase the utilization of nutrients in the gut and improve FCR.

Immunity assay

Increased immune responses have been reported with the use of probiotic [37, 48] and herbal extracts [26, 42] in diets, which is in agreement with the results of current study. Researchers have shown that diets containing probiotics increase immune response via enhancement of the formulating bacteria on an acquired immune response exerted by T and B

[DOR: 20.1001.1.2717204.2011.10.37.10.1]

lymphocytes [34]. Immune system stimulation by probiotics may be due to increase of T-cells, phagositosic cells and serum protein levels [20]. Christensen et al. [11] suggested that these effects were mediated by cytokines secreted by immune system cells stimulated with probiotic bacteria. Indirect effect of probiotics may occur via changing the microbial population of the lumen of gastrointestinal tract. Probiotics increase in gram positive bacteria such as, *lactobacillus* and *bifidobacteria* that improve immune response [34].

It has been proved that herbal extracts increase anti-body titration against SRBC [42]. Cook and Samman [12] noted that herbal extracts stimulate immune response by increasing vitamin C activity. Sangrovit® is known to have immunomodulatory effects [9]. It has been reported that Sangrovit® stimulates phagocyte activity and thus promotes host protective responses [26]. Researchers have shown that adding organic acids to broiler diets increase immunity response [53]. It has also been suggested that this increase is due to stimulation or activation of immune cells by organic acids. Organic acids can decrease intestinal pH, and cause enhancement of gut characteristics and immune response. Khovidhunkit et al. [36] showed that antibiotics restrain gram-positive bacteria that stimulate the immune system. Therefore, antibody titration was diminished by use of antibiotic which was confirmed by the results of this study.

Serum lipides

Serum cholesterol and triglyceride levels also were reduced by use of herbal extracts in this study which agrees with results of Sakine et al. [55] who show that use of herbal extracts can reduce cholesterol and triglyceride levels of blood. Gudev et al. [26] proved that Sangrovit® can reduce cholesterol and triglyceride levels of serum in pigs. It has been hypothesized that the increased deconjugation of bile acids, results in greater excretion of bile acids from the intestinal tract. The increased excretion of bile acids stimulates the replacement of bile acids from cholesterol, thus reducing plasma cholesterol level [14].

Reduction of blood cholesterol and triglyceride levels with probiotics has been reported by Santoso et al. [58] and Ignatoval et al. [30]. Gilliland et al. [24] believe that *lactobacillus* can absorb cholesterol in their cell walls and therefore reduce cholesterol in blood. It has been reported that some gram-positive bacteria such as *Lactobacillus* and *Bifidobacteria* cause deconjugation of bile acids; this causes reduction of blood cholesterol. On the other hand, probiotics cause increase in gram-positive bacteria. These reports are in agreement with the results of this study. Also, probiotics lead to reduction in acetyl Co-A carboxylase in liver and tissue. Lipogenesis reduction in liver can lead to reduction in serum lipids [36]. Organic acids in diet are known to cause an increase in T_3 hormones. This hormone increases metabolism in birds, thus can decrease serum lipids [13]. Virginiamycin could cause an increase in blood cholesterol due to its restraining effect on gram-positive bacteria [36]. Results of recent experiment confirmed this finding.

Microflora population

Probiotics improve intestinal microbial balance of animals [20]. Chichlowisk et al. [10] proved that probiotics increased metabolic efficiency by inducing changes in intestinal physiology and metabolism. Probiotic cultures of *lactobacillus* sp. cause a challenge for pathogenic bacteria for nutrients. They reduce nutrients availability for harmful bacteria nutrients by pathogenic bacteria and

[DOR: 20.1001.1.2717204.2011.10.37.10.1]

reduce nutrients availability for harmful bacteria and increase *lactic acid* bacteria colonies in intestine [45]. Djouvinov et al. [15] showed that adding probiotics to ducks feed increased the *lactic acid* bacteria in cecum compared to control group. The benefit of *lactic acid* bacteria seems to be due to its production of bacteriocins, which 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*). Present study proves that probiotic has increased *lactic acid* bacteria, reduced number of coliforms and total aerobic bacteria counts in intestinal contents.

It is known that plant antibacterial activities are due to their lipophilic activites [17]. The mechanism of medicinal plant for decreasing bacteria is adhesion and thrust of bacterial membrane which prevents activation of bacterial enzymes [65]. Gudev et al. [26] showed that pigs fed with Sangrovit® had a higher lizozim amount in blood serum. Lizozim can breakdown cell wall of some bacteria. On the other hand, Sangrovit® is a commercial product containing benzophenanthridine alkaloid (QBA) and mostly sanguinarine. QBA's have antimicrobial properties [40]. It is known that sanguinarine has an inhibiting effect on harmful bacteria in the intestine [41]. Reduction on coliforms and total aerobic bacteria counts in crop, ileum and cecum can be seen in present study.

Antibiotics can lower population of harmful bacteria and enhance population of useful bacteria in the intestine [4]. It has been proved that antibiotics decrease *lactic acid* bacteria in intestine [4]. In this experiment use of antibiotic caused a reduction on coliforms and total aerobic bacteria in different part of the gut. Moreover, reduction in *lactic acid* bacteria was observed. These results are in agreement with previous studies [52, 28].

Organic acids are known to reduce pH of intestine and cecum, and thus could reduce harmful bacteria sensitive to low pH [1]. In current study using organic acid termin-8® reduced harmful bacteria and increased *lactic acid* bacteria, these results are in agreement with other studies [68].

Intestinal morphology

Intestinal morphology characteristics are affected by dietary treatments. In this study the results showed that the use of probiotics improved intestinal morphology characteristics, which this reaction can lead to increase feed utilization and improve performance. In a study conducted by Chichlowisk et al. [10] it has been stated that a probiotic containing *lactobacilli Bifidobacterium thermophilum* and *Enterococcus faecium* increased the jejunum villous height and decreased the villous depth compared with control group. Samanta et al. [57] showed that adding probiotics to broilers feed for 7 days increases villous height compared to control group. It has been reported that probiotics increase Short Chain Fatty Acids (SCFA) and decrease the production of ammonium [54]. Moreover, Garcia et al. [21] showed that using medicinal plants in feed causes a higher villous in chickens. They suggest that medicinal plants decrease the total harmful bacteria in the intestinal wall and cause a reduction in production of toxic compounds and damage to intestinal epithelial cells. This function could lead to a conversion in intestinal morphology. In another study Sangrovit® significantly increased SCFA's in intestine [33]. These fatty acids could lower pH of the small intestine and improve microbial population of gut. Miles et

[DOR: 20.1001.1.2717204.2011.10.37.10.1]

al. [43] reported that broilers fed antibiotic (virginiamycin) supplemented diet, had a thinner mucosa, shorter villous height and crypt depth. Antibiotics decline harmful bacteria, inhibit the destruction of villous and decreases reconstruction of the lumen. The results in this study are in agreement with other researches [43].

Researchers have shown that organic acids can reduce the intestinal lumen pH and increase anti-bacterial enzymes produced by some bacteria, so villous height may be increased [51]. Moreover, organic acids reduce amount of pathogenic bacteria in the small intestine wall and decreases production of toxic compounds which cause changes in the morphology of the intestine of broiler chicks and in consequence prevent destruction and damage to intestinal epithelial cells [21].

Conclusion

It has been suggested that antibiotic alternatives cause reduce pathogenic bacteria in digestive tract of broiler chickens which can help to improve intestinal health of these birds. Moreover, the above mentioned feed additives reduce the levels of serum lipids and induce broilers immune response. A beneficial effect of additives on the villous parameters of chickens could be due to better utilization of feed and improve the microflora and reduce harmful bacteria of the gut. According to results of this experiment it can be recommended that probiotics additives, herbal extracts and organic acids can be used as antibiotic alternatives in broilers feed.

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