J. Agr. Sci. Tech. (2012) Vol. 14: 799-810

Effects of Yarrow (*Achillea millefolium* L.), Antibiotic and Probiotic on Performance, Immune Response, Serum Lipids and Microbial Population of Broilers

S. Yakhkeshi[1], S. Rahimi[1*], and H. R. Hemati Matin[1]

ABSTRACT

This study was conducted to investigate the effects of the medicinal plant yarrow (*Achillea millefolium* L.), a probiotic (Primalac) and an antibiotic (virginiamycin) on gastrointestinal tract (GIT) characteristics, microbial populations, immune response, serum lipids and growth performance of broiler chickens. A total of 250 one-day old male broilers (Ross 308) were randomly allocated to 5 treatments, 5 replicates with 10 birds in each in a completely randomized design. Experimental treatments included the control, yarrow powder at two different concentrations (1.5 and 3% of diet), Primalac (0.1% of diet) and virginiamycin (15 ppm). The highest feed conversion ratio (FCR) was observed in the control while the lowest FCR was seen in the virginiamycin group at 42 days of age (P< 0.05). Moreover, the highest body weight gain (BWG) was observed in the virginiamycin group while the lowest value was related to the control animals (P< 0.05). Carcass yields were not different between treatments (P> 0.05). Relative weights of breast and thigh were similar among all treatments (P> 0.05). Relative weights of bursa Fabricius, spleen and primary immune response (total titer, IgG and IgM) against sheep red blood cells (SRBC) were not affected by treatments. The serum cholesterol, triglyceride as well as high and low density lipoprotein (LDL and HDL) levels were different among treatments (P< 0.05). The lowest concentrations of the mentioned parameters were obtained in the group supplemented with 3% of yarrow (P< 0.05). The highest and lowest antibody titers (secondary immune response) against SRBC were observed by the yarrow (3%) and antibiotic supplementations, respectively (P< 0.05). The highest lactic acid bacteria (LAB) counts were detected in the crop, ileum and cecum of the Primalac group (P< 0.05). Inclusion of virginiamycin and yarrow (3%) caused a significant decrease in coliforms and total aerobic bacteria counts in crop, ileum and cecum (P< 0.05). The results of this study showed that the administration of yarrow (3%) can reduce the levels of serum lipids and boost the immune response in broilers. Moreover, it led to reduced pathogenic bacteria population in the GIT which could help to improve intestinal health and well being of poultry. It is proposed that yarrow can be used as an antibiotic alternative.

Keywords: Broiler performance, Immune response, Microbial population, Primalac, Virginiamycin, Yarrow.

INTRODUCTION

Antibiotics as growth promoters have been used to control intestinal health, to alter microbial population and to improve growth and feed efficiency in poultry for several years (Gibson and Fuller, 2000). However, antibiotic resistance and unreliable antibiotic therapy in poultry (Joerger, 2002) have led to a ban on antibiotic use in many countries (Patterson and Burkholder, 2003). Increasing investigations regarding alternatives to antibiotics were widely carried out to achieve the best growth performance (Jones *et al.,* 2003). Numerous additives are used or proposed as effective means to reduce or eliminate pathogens or to improve growth and FCR (Joerger, 2002). Probiotics (Awad *et al.,* 2006), prebiotics (Biggs *et al.,* 2007), organic acids (Gunal *et*

[1] Department of Poultry Science, Faculty of Agriculture, Tarbiat Modares University, P. O. Box: 14115-336, Tehran, Islamic Republic of Iran.

[*] Corresponding author; e-mail: rahimi_s@modares.ac.ir

al., 2006), enzymes (Viveros *et al.,* 1994) and medicinal plants (Sakine *et al.,* 2006) are extensively used in poultry feed. It is well documented that effects of these feed additives are mediated by intestinal microflora (Joerger, 2002). Important characteristics of probiotics and prebiotics are the increase of animal resistance to diseases and the improvement of feed efficiency without any residual in the poultry tissue (Silva *et al*., 2000). In addition, probiotics are used not only as a growth promoter, but also they enhance the immune system and have protective effects against many diseases (Gibson and Fuller, 2000). Moreover, some probiotic strains are able to reduce absorption of bile acids from intestine (Doncheva *et al.,* 2002) and to decrease LDL and VLDL levels significantly. Although, lipoproteins have a role in the elimination of cholesterol, no changes have been reported in this regard (Mohan *et al.,* 1996). On the other hand, probiotics produce short-chain fatty acids and reduce cholesterol synthesis in the liver whereby reducing host blood cholesterol. Nevertheless, results of probiotics application in poultry are very variable (Denli *et al*., 2003). Other antibiotic alternatives are medicinal plants, which are being used as feed supplements to improve growth performance, to manipulate gut functions and microbial habitat of domestic animals (Panda *et al.,* 2000). Previous studies have demonstrated the positive effects of herbal supplements on production performance and carcass quality (Schleicher *at al*., 1998; Guo *et al*., 2004b; Tekeli *at al*., 2006; 2008). A variety of herbal supplements have been widely used to maintain and improve health of humans (Freeman and Kodera, 1995) and birds (Gardzielewska *et al.,* 2003). They can improve immune system (Mathivanan and Kalaiarasi, 2007), reduce blood cholesterol (Sakine *et al*., 2006) and/or improve growth performance and feed efficiency (Garca *et al.,* 2007).

Yarrow (*Achillea millefolium* L.) is one of the antibiotic alternatives with proven antifungal and antimicrobial effects (Omidbaygi, 2004). Yarrow is a flowering plant belonging to the family *Asteraceae*. It is used for the treatment of many digestive disorders and allergy; essential oils of yarrow have anti-inflammatory effects (Omidbaygi, 2004).

Although there are many inconsistent results regarding substitution of probiotic and medicinal plants for antibiotic and clarifying roles of these additives in poultry production, some of these additives have been reported to have a big potential to replace antibiotics. Therefore, the present study was carried out to determine whether yarrow (*Achillea millefolium*) would influence the growth performance, carcass characteristics, digestive system development, intestinal microflora, immune response, and serum lipids of broilers. Moreover, in this study, comparative investigation of yarrow, a probiotic (Primalac) or the antibiotic virginiamycin supplementation was done.

MATERIALS AND METHODS

Birds and Diets

A total of 250 one-day-old male broilers (Ross 308) were randomly allocated to 5 treatments, 5 replicates with 10 birds in each. Treatments included the control, virginiamycin (Phibro, USA) (15 ppm), Primalac (Star–Labs, USA; 0.1% of diet), and two different levels of yarrow (1.5 and 3% of diet). The application period lasted 42 days. The birds were kept in floor pens. Feed and water were provided *ad libitum* throughout the study. Lighting schedule was 23 L/1 D. 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 (1994) which is presented in Table 1**.** Feed intake (FI), BWG and FCR were measured. The experiment

Table1 . Diet formulation and calculated chemical composition [a].

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	4.20	8.09	14.68
Soybean oil	1.10	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 [b]	0.25	0.25	0.25
Mineral permix [c]	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 %	0.90	0.82	0.72

[a] Virginiamycin, yarrow and primalac were add to the basal diet at 15 ppm, 1.5 and 3% and 0.1 % to make the respective diets for each experiment, respectively.

[b] 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).

[c] 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); 0.15mg of selenium (NaSeO3); 80 mg of zinc (ZnSO4_H2O); and 0.5 mg of cobalt (CoSO4).

was approved by Animal Care Committee of Tarbiat Modares University, Tehran, Iran.

Gut and Carcass Yield Characteristics

Ten birds from each replicate were randomly selected and sacrificed by cervical dislocation at 42 days of age. Weights of crop, gizzard, liver and length of different segments of intestine, as well as relative weights of lymphoid organs (spleen and bursa Fabricius) were measured. Moreover, carcass weight and characteristics (breast, thighs, and wings) were measured.

Immunity and Blood Parameters Assay

Two injections of SRBC antigen were done intramuscularly for the evaluation of immune system responses at 21 and 35 days. Two birds from each replicate were randomly selected and blood samples were taken via wing vein at 28 and 42 days. Thereafter, anti-body titration against SRBC was performed by hemaglutination inhibition (HI) test, after which immunoglobulin M and G (IgM and IgG) were determined by use of 2-Mercaptoethanol (Wegmann *et al.,* 1966).

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 a spectrophotometer (UV) at 546 nm wavelength.

Microbial Sampling and Incubation

On day 42 of the experiment, two birds from each replicate were killed by CO_2

inhalation and crop, ileum and cecum contents were collected. Contents were gently removed into sterile sampling tubes and immediately transferred on ice to the laboratory. The contents of the mentioned segments were used for microbial study. Serial dilutions of 1 g sample (10^{-4} to 10^{-7}) were made. Thereafter, selective media of Plate Count Agar (Merck, Germany), De Man Rogosa Sharpe Agar (Merck, Germany) and MacConkey Agar (Merck, Germany) were inoculated to detect the total counts of aerobic bacteria, lactic acid bacteria (LAB) and coliforms, respectively. Microbial populations of total aerobic bacteria and coliforms were counted after aerobic incubation at 37°C for 24 hours and LAB after aerobic incubation at 37°C for 48 hours (Witkamp, 1963).

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 (SAS, 2004). Duncan's multiple range test was used to compare means (P< 0.05).

RESULTS

Growth Performance, Gut Parameters and Carcass Yield

The effects of dietary treatments on performance are shown in Table 2. No significant differences were found between treatments in FI at 1-21, 21-42 and 1-42 days of age (P> 0.05). Moreover, no significant differences were obtained in WG at 1-21 days of age (P> 0.05). A significant increase in WG was observed by virginiamycin supplementation at 21- 42 and 1-42 days of ages (P< 0.05) which did not differ compared to yarrow (1.5 and 3 %) and Primalac group (P> 0.05) but were still higher than in the control (P< 0.05). No significant differences in FCR were found between treatments at 1-21 days of age (P> 0.05). The addition of virginiamycin to the diet caused a significant decrease in FCR at 22-42 and 1-42 days of age compared to the control (P< 0.05). No differences in FCR between yarrow (1.5 and 3%) and Primalac treatments were detected (P> 0.05).

The effects of dietary treatments on carcass characteristics are given in Table 3. Results revealed no significant differences between treatments in any measured parameters (P> 0.05). The effects of dietary

Table 2. Broilers performance in response to various dietary treatments.

Measurement	Treatments					SEM	P-value
	Control	Yarrow (1.5%)	Yarrow (3%)	Virginiamycin	Primalac		
Feed intake (g)							
1-21 d	1098	1123	1124	1110	1137	21.03	0.910
22-42 d	3260	3309	3274	3315	3265	39.98	0.992
1-42 d	4359	4432	4399	4426	4403	43.65	0.991
Weight gain (g)							
1-21 d	770	820	818	825	826	28.90	0.283
22-42 d	1512^{b}	1646^{ab}	1581^{ab}	1733^{a}	1633^{ab}	34.21	0.243
1-42 d	2283^{b}	2466^{ab}	2399^{ab}	2559^{a}	2487^{ab}	46.75	0.169
Feed conversion ratio							
1-21 d	1.42	1.37	1.37	1.37	1.34	0.011	0.274
22-42 d	2.16^{a}	2.01^{ab}	2.07^{ab}	1.88^{b}	2.03^{ab}	0.035	0.143
1-42 d	1.91^{a}	1.79^{ab}	1.83^{ab}	1.71^{b}	1.77^{ab}	0.023	0.086

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

Table 3. The effects of various treatments on carcass characteristics of broilers.

Treatments	Carcass weight	Carcass yield	Abdominal fat	Breast	Thighs	Wings
	g	%BW			%Carcass	
Control	2428.05	60.01	1.12	28.51	17.12	8.91
Yarrow (1.5%)	2372.76	60.44	1.35	30.02	17.48	8.74
Yarrow (3%)	2533.33	60.18	1.38	29.36	17.70	8.71
Virginiamycin	2412.88	60.31	1.60	29.27	18.98	9.57
Primalac	2398.72	59.57	1.52	31.43	17.48	9.26
SEM	42.89	59.57	0.122	0.396	0.241	0.188
P-value	0.848	0.415	0.834	0.183	0.105	0.611

SEM, Standard Means of Errors.

treatments on relative length of different segments of intestine are presented in Table 4. None of dietary treatments produced significant differences in any of the measured parameters (P> 0.05).

Immunity Assay

The results of dietary treatments on immune response of broilers are presented in Table 5. No significant differences were found between treatments in the relative weights of bursa Fabricius and spleen (P> 0.05). The primary immune response against SRBC was not affected by the treatments (P> 0.05), but the secondary immune response was affected significantly (P< 0.05). The highest total antibody titers against SRBC was related to yarrow (3%) (P< 0.05) compared to other treatments. No significant differences were seen between other treatments (P> 0.05). Moreover, yarrow (3%) caused significant increases in IgG and IgM amounts when compared to the control (P< 0.05).

Blood Parameters

The effects of treatments on triglyceride, cholesterol, and high and low density lipoproteins are given in Table 6. The highest and lowest serum cholesterol levels were attained by antibiotic and yarrow (3%) groups, respectively (P< 0.05). In addition, the highest and lowest serum triglyceride and high density lipoprotein concentrations were obtained by virginiamycin and yarrow (3%), respectively (P< 0.05). Also, the highest and lowest low density lipoprotein levels of serum were achieved by virginiamycin and yarrow (3%), respectively (P< 0.05).

Microbial Populations

The effects of dietary treatments on microbial population of crop, ileum and cecum are shown at Table 7. The lowest

Table 4. Length and relative length of different segments of intestine in response to dietary treatments.

Treatments	Length (cm)			Relative length			Relative weight		
	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum	Liver	Gizzard	Crop
Control	29.75	83.75	83.30	0.0152	0.042	0.042	0.025	0.023	0.0071
Yarrow (1.5%)	29.50	83.25	84.30	0.0147	0.042	0.043	0.026	0.019	0.0049
Yarrow (3%)	30.50	84.86	85.63	0.0152	0.043	0.041	0.026	0.021	0.0062
Virginiamycin	28.20	82.10	82.40	0.0147	0.041	0.043	0.023	0.021	0.0065
Primalac	28.75	82.60	82.80	0.0147	0.043	0.042	0.023	0.021	0.0059
SEM	0.485	0.553	0.532	0.001	0.004	0.004	0.0005	0.0005	0.0003
P-value	0.672	0.621	0.336	0.855	0.821	0.643	0.171	0.322	0.318

SEM, Standard Means of Errors.

Table 5. The effects of dietary treatments on immune response of broilers.

Treatments	Post first immunization			Post second immunization			Bursa	Spleen
	Total titer	IgG	IgM	Total titer	IgG	IgM	%BW	
Control	3.44	2.35	1.08	4.09^{b}	2.97^{b}	1.12^{b}	0.180	0.128
Yarrow (1.5%)	4.05	2.84	1.20	4.38^{b}	3.03^{ab}	1.35^{ab}	0.174	0.129
Yarrow (3%)	4.01	2.68	1.32	5.58^{a}	4.06^{a}	1.52^{a}	0.174	0.129
Virginiamycin	3.94	2.76	1.18	4.05^{b}	2.91^{b}	1.14^{b}	0.182	0.131
Primalac	3.91	2.71	1.20	4.72^{a}	3.49^{ab}	1.22^{ab}	0.145	0.137
SEM	0.128	0.110	0.037	0.174	0.151	0.057	0.010	0.004
P-value	0.628	0.404	0.742	0.0001	0.0001	0.454	0.838	0.972

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

Table 6. The effects of treatments on some blood parameters of broilers.

Treatments	TG ^{a}	CH ^{b}	HDL^{c}	LDL ^{d}
	(Mg dl^{-1})			
Control	51.66^{ab}	139.00^{a}	60.00^{a}	63.66^{ab}
Yarrow (1.5%)	50.00^{ab}	127.33^{ab}	55.66^{ab}	61.33^{ab}
Yarrow (3%)	42.66^{b}	118.33^{b}	50.33^{b}	57.00^{b}
Virginiamycin	57.33^{a}	143.00^{a}	64.00^{a}	66.00^{a}
Primalac	48.00^{ab}	132.33^{ab}	57.00^{ab}	57.66^{ab}
SEM	1.92	2.96	1.62	1.31
P-value	0.177	0.246	0.057	0.061

^{abc}Means in columns with different superscripts were significantly differ (P<0.05). SEM, Standard Means of Errors. ^{a} Triglyceride; ^{b} Cholesterol; ^{c} High density lipoprotein; ^{d} Low density lipoprotein.

Table 7. The effects of dietary treatments on crop and intestine microbial population of broilers.

Treatment	Bacterial population								
	crop			ileum			cecum		
	TA^{a}	LAB^{b}	COF^{c}	TA	LAB	COF	TA	LAB	COF
	(Log_{10} cfu/g of DM)								
Control	8.20^{a}	6.16^{b}	9.70^{a}	8.49^{a}	6.39^{c}	8.68^{a}	8.75a^{a}	7.38^{b}	9.11^{a}
Yarrow (1.5%)	7.86^{a}	6.31^{b}	9.48^{a}	8.29^{a}	6.73^{cb}	8.33^{b}	8.71^{ab}	7.80^{ab}	8.21^{bc}
Yarrow (3%)	7.18^{bc}	6.33^{b}	8.78^{b}	7.76^{b}	6.62^{b}	7.41^{c}	8.30^{ab}	7.70^{ab}	8.34^{b}
Virginiamycin	6.78 ^{c}	5.53^{c}	8.46^{c}	7.27^{c}	5.93^{c}	7.03^{c}	7.66^{c}	6.31^{b}	7.91^{c}
Primalac	7.69^{bc}	6.89^{a}	9.10^{b}	8.18^{a}	7.21^{a}	8.06^{b}	8.47^{ab}	8.10^{a}	8.92^{a}
SEM	0.118	0.105	0.107	0.103	0.098	0.140	0.095	0.143	0.106
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

^{abc}Means in columns with different superscripts were significantly differ (P<0.05). SEM, Standard Means of Errors.
^{a} Total aerobic; ^{b} lactic acid bacteria; ^{c} Total coliforms**.**

total aerobic counts in crop was detected in virginiamycin supplemented animals (P< 0.05) whereas Primalac and yarrow (3%) groups showed still lower counts compared to the control (P> 0.05). The highest and lowest counts of LAB were reached by Primalac and virginiamycin groups in crop (P< 0.05). Thus, virginiamycin treatment caused a significant reduction in LAB in this segment compared to the control (P< 0.05). Inclusion of virginiamycin and yarrow (3%) to diets led to significant decreases in coliforms and total aerobic bacteria counts in crop, ileum and cecum (P< 0.05).

DISCUSSION

Performance, Carcass Yield and Organ Weight

It is proposed that antibiotics reduce the competition for microbial nutrients in the host and thereby increase the availability of nutrients (Vukic Vramjes and Wenk, 1995) by reducing pathogenic bacteria (Miles *et al*., 2006) and their toxins in the intestine. These reactions can improve FCR and increase BWG (Bafundo and Bywater, 2003; Ferket *et al*., 2003) which agree with the results of this study. On the other hand, antibiotics affect the thickness of epithelium layer and result in increased absorption efficiency of nutrients in the intestine (Miles *et al*., 2006) that can lead to better performance.

Any factor that increases the activity of an organ above threshold levels can lead to increases in organs weight and length by hypertrophy and hyperplasia of the related organs. It seems that the use of feed additives in this study did not induce organs activation and increase in organs weight and length which agrees with results of Gunes *et al*. (2001) and Cabuk *et al*. (2006). Also, the results of the present study are similar to those reports of other researchers (Denli *et al.,* 2003; Zhang *et al.,* 2005; Pelicano *et al.,* 2005) who noted that the use of feed additives (essential oils, probiotics and antibiotics) had no effect on carcass and relative organs weights. In another experiment, Gong *et al.* (2001) illustrated that carcass yield and relative weights of organs in broilers were mainly affected by genetic factors and less influenced by nutritional factors. Although, Leeson (1984) reported that carcass yields were improved by the use of antibiotics, various studies indicated that probiotics, prebiotics and antibiotics have no effects on carcass yield (Mohan *et al.,* 1996; Denil *et al.,* 2003; Pelicano *et al.,* 2005). Pelicano *et al.* (2004), Lodi *et al.* (2000), Demir *et al.* (2003) and Zhang (2005) reported that addition of probiotics, prebiotics, antibiotics and medicinal plants had no effect on abdominal fat which was also observed in this study. Feed additives may interact with fat digestion and absorption which can affect abdominal fat but it seems that the mentioned feed additives did not have any interference with these phenomena.

Immunity Assay and Some Blood Parameters

Increased immune responses have been reported with the use of probiotics in diets (Panda *et al*., 2000; Cotter *et al*., 2000; Fuller, 1992) which is in agreement with the results of our study. Immune system stimulation by probiotics may be due to the increase of T-cells, phagocytic cells and serum protein levels (Fuller, 1989). Mathivanan and Kalaiarasi (2007) showed that medicinal plants increased anti-body titration against SRBC more than the antibiotic (virginiamycin). The application of medicinal plants resulted in immune system stimulation e.g. via increasing vitamin C activity (Cook and Samman, 1996) which is in agreement with results of this experiment. The roles of endogenous enzymes and intestinal microflora effects caused by medicinal plants need more investigations. Also, antibiotics restrain the gram-positive bacteria (Humphrey *et al.,* 2002; Khovidhunkit *et al.,* 2004) that stimulate the immune system. Therefore, antibody titration is diminished by the use of the antibiotic which was confirmed by the results of this study. Some bacterial strains among lactobacilli and bifidobacteria can reduce blood cholesterol contrary to antibiotics (virginiamycin) which reduce gram-positive bacteria leading to an increase in blood cholesterol in groups fed with antibiotics (Humphrey *et al.,* 2002; Khovidhunkit *et al.,* 2004). In the group which was fed with medicinal plant (yarrow, 3% of diet) significant cholesterol reduction was obtained which is in agreement with the results of Craig (1999). Blood cholesterol

and triglyceride levels were also reduced in the study of Sakine *et al.* (2006) using a medicinal plant. It is proposed that medicinal plants cause a reduction in liver 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-COA) (key enzyme in cholesterol synthesis regulation; Middleton and Hui, 1982; Yu *et al.,* 1994). On the other hand, cholesterol reduction may be due to bile acids break down that subsequently inhibit re-synthesis of cholesterol. Santoso *et al.* (1995) reported reduction of blood triglyceride levels after probiotic supplementation similar to the observation made in this study. It seems that probiotics lead to a reduction in acetyl Co-A carboxylase (limited enzyme in fatty acids synthesis) in liver and tissue. Lipogenesis reduction in liver can lead to reduction in HDL and triglycerides (Santoso *et al.,* 1995; Khovidhunkit *et al.,* 2004).

Microbial Populations

Probiotics beneficially affect the host animal by improving its intestinal balance (Fuller, 1989). Chichlowisk *et al.* (2007) provided data suggesting that Primalac increased metabolic efficiency via changes in intestinal physiology and metabolism. In this study, selected probiotic cultures of *Lactobacillus* sp. competed with pathogenic bacteria for nutrients and reduced nutrients availability for detrimental bacteria. It is reported that antibiotics can increase the population of beneficial bacteria in the intestine while lowering the population of *E. coli* (Baurhoo, 2007). In this experiment, use of the antibiotic caused a reduction in coliforms and total aerobic bacteria counts in crop, ileum and cecum which was similar to the results of other researchers (Rosen, 1995; Jensen, 1993; Guo *et al.,* 2004a). Many plant oils and extracts have been reported to have antimicrobial properties (Hoffman, 1987; Lawless, 1995). It is proposed that plant antibacterial properties are related to their lipophilic characters (Farag *et al*., 1989c). The major mechanism of medicinal plants is adhesion and thrust of bacterial membrane which inhibits bacterial enzymes activation (Shapiro and Guggenheim, 1995; Stiles *et al.,* 1995). These reactions can reduce pathogenic populations in the intestine which was also seen in the present study by reducing coliforms and total aerobic bacteria counts in crop, ileum and cecum.

CONCLUSIONS

The findings of the present study showed that yarrow (3% of diet) can reduce the levels of serum lipids and induce immune response in broilers. Moreover, the supplementation of yarrow led to the reduction of pathogenic bacteria in the digestive tract which can help to improve intestinal health. However, yarrow does not raise growth performance to the same level as the antibiotic virginiamycin. It is probable that yarrow has partial antibacterial properties as well as an ability to decrease plasma lipids. Therefore, yarrow can be used as an antibiotic alternative in poultry production.

REFERENCES

1. Awad, W. A., Bohm, J., Razzazi-Fazeli, E., Ghareeb, K. and Zentek, J. 2006. Effect of Addition of a Probiotic Microorganism to Broiler Diets Contaminated with Deoxynivalenol on Performance and Histological Alterations of Intestinal Villi of Broiler Chickens. *Poult. Sci.*, **85**: 974–979.
2. Bafundo, K. W., Cox, L. A. and Bywater, R. 2002. Review Lends Perspective to Recent Scientific Findings on virginiamycin, Antibiotic Resistance Debate. *Feed Stuffs,* **75(3)**: 26-27.
3. Baurhoo, B., Hillip, L. P. and Ruiz-Feria, C. A. 2007. Effects of Purified Lignin and Mannan Oligosaccharides on Intestinal Integrity and Microbial Populations in the Ceca and Litter of Broiler Chickens. *Poult. Sci.*, **86**: 1070–1078

4. Biggs, P. and Parsons, C. M. 2007. The Effects of Several Oligosaccharides on True Amino Acid Digestibility and True Metabolizable Energy in Cecectomized and Conventional Roosters. *Poult. Sci.*, **86**: 1161–1165.
5. Cabuk, M., Bozkurt, M., Alcicek, A., Akbas, Y. and Kucukyimaz, K. 2006. Effect of a Herbal Essential Oil Mixture on Growth and Internal Organ Weight of Broiler from Young and Old Breeder Flock. *S. Afr. J. Anim. Sci.*, **36(2)**: 324-354.
6. Chichlowisk, M., Croom J., McBride, B. W., Daniel, L., Davis, G. and Koci, M. D. 2007. Direct-fed Microbial PrimaLac and Salinomycin Modulate Whole-body and Intestinal Oxygen Consumption and Intestinal Mucosal Cytokine Production in the Broiler Chick. *Poult. Sci.*, **86**: 1100–1106.
7. Cook, N. C. and Samman, S. 1996. Flavonoids-chemistry, Metabolism, Cardioprotective Effects, and Dietary Sources. *J. Nutr. Biochem.*, **7**: 66-76.
8. Craig, W. J. 1999. Health-promoting Properties of Common Herbs. *Am. J. Clin.*, **70(l)**: 491-499.
9. Demir, E., Sarica, S., Ozcan, M. A. and Suicmez, M. 2003. The Use of Natural Feed Additives as Alternatives for Antibiotic Growth Promoter in Broiler Diets. *Br. Poult. Sci*., **44**: 44-45.
10. Denli, M., Okan, F. and Celic, K. 2003. Effect of Dietary Probiotic, Organic Acid and Antibiotic Supplementation to Diets on Broiler Performance and Carcass Yield. *Pak. J. Nutr.*, **2**: 89-91.
11. Doncheva, N. I., Antov, G. P., Softova, E. B. and Nyagolov, Y. P. 2002. Experimental and Clinical Study on the Hypolipidemic and Antisclerotic Effect of *Lactobacillus bulgaricus* Strain GB N^{1} (48). *Nutr. Res.*, **22**: 393-403.
12. Farag, R. S., Daw, Z. Y., Hewed, F. M. and El-Barory, G. S. A. 1989. Antimicrobial Activity of Some Egyptian Spice Essential Oils. *J. Food Protec.*, **52**: 665-667.
13. Ferket, P. R., Parks, C. W. and Grimes, J. L. 2003. Benefits of Dietary Antibiotic and Mannan Oligosaccharides Supplementation for Poultry. M*ulti-State Poultry Meetin*g, May 14-16, USA.
14. Freeman, F. and Kodera, Y. 1995. Garlic Chemistry: Stability of S-(2-propenyl) 2-propene-1-sulfinothioate (Allicin) in Blood, Solvents, and Stimulated Physiological Fluids. *J. Agric. Food. Sci.,* **43**: 2332–2338
15. Fuller, R. 1989. Probiotics in Man and Animals: A Review. *J. Appl. Bacteriol.*, **66**: 365-378.
16. Fuller, R. 1992. The Effect of Probiotics on the Gut Microecology of Farm Animals. In: "*The Lactic Acid Bacteria in Health and Disease*", (Ed.): Wood, J. B.. Elsevier Applied Science, New York, NY, PP. 171–192.
17. Garca, V., Catala-Gregori, P., Hernandez, F., Megıas, M. D. and Madrid, J. 2007. Effect of Formic Acid and Plant Extracts on Growth, Nutrient Digestibility, Intestine Mucosa Morphology, and Meat Yield of Broilers. *J. Appl. Poult. Res.*, **16**: 555–562.
18. Gardzielewska, J., Pudyszak, K., Majewska, T., Jakubowska, M. and Pomianowski, J. 2003. Effect of Plant-supplemented Feeding on Fresh and Frozen Storage Quality of Broiler Chicken Meat. *J. Polish. Agric. Univ.*, **6(2)**. Animal Husbandry Series of Electronic: http://www.ejpau.media.pl/series/volume6/issue2/animal/art-12.html.
19. Gibson, G. R. and Fuller, R. 2000. Aspects of *In vitro* and *In vivo* Research Approaches Directed toward Identifying Probiotics and Prebiotics for Human Use. *J. Nutr.*, **130**: 391–395.
20. Gong, J., Forster, R. J., Yu, H., Chambers, J. R., Sabour, P. M., Wheatcroft, R. and Chen, S. 2002. Diversity and Phylogenetic Analysis of Bacteria in the Mucosa of Chicken Ceca and Comparison with Bacteria in the Cecal Lumen. *FEMS Microbiol. Lett.*, **208**: 1–7.
21. Gunal, M., Yayli, G., Kaya, O., Karahan, N. and Sulak, O. 2006. The Effects of Antibiotic Growth Promoter, Probiotic or Organic Acid Supplementation on Performance, Intestinal Microflora and Tissue of Broilers. *Poult. Sci.*, **5(2)**: 149-155.
22. Gunes, H., Cerit, H. and Altinel, A. 2001. Effect of Organic Acid, Probiotic and Antibiotic on Performance and Carcass Yield of Broilers. *Anais da XXXV Reuniao*

da Sociedade Brasileira de Zootecnia, 302-308.
23. Guo, F. C., Williams, B. A., Kwakkel, R. P., Li, H. S., Li, X. P., Luo, J. Y., Li, W. K. and Erstegen, M. W. A. 2004. Effects of Mushroom and Herb Polysaccharides, as Alternatives for an Antibiotic, on the Cecal Microbial Ecosystem in Broiler Chickens. *Poult. Sci.*, **83**: 175–182.
24. Guo, F. C., Kwakkel, R. P., Soede, J., Williams, B. A. and Verstegen, M. W. 2004. Effect of a Chinese Herb Medicine Formulation, as an Alternative for Antibiotics, on Performance of Broilers. *Br. Poult. Sci.*, **45**(6): 793-797.
25. Hoffman, D. L. 1987. *The Herb User's Guide*. Thorsons Publishing Group, Wellingborough, UK, 240P.
26. Humphrey, B. D., Koutsos, E. A. and Klasing, K. C. 2002. Requirement and Priorities of the Immune System for Nutrients. In: "*Nutritional Biotechnology in the Feed and Food Industries*", (Eds): Jacques, K. A. and Lyons, T. P.. *P. A. Annu. Sym.,* PP. 69-77.
27. Jensen, B. B. 1993. The possibility of Manipulating the Microbial Activity in the Digestive Tract of Monogastric Animals. *44th Annual Meeting, European Association for Animal Production,* Denmark, PP. 20
28. Joerger, R. D. 2002. Alternatives to Antibiotics: Bacteriocins, Antimicrobial Peptides and Bacteriophages. *Poult. Sci.*, **82**: 640–647.
29. Jones, F. T. and S. C. Ricke. 2003. Observations on the History of the Development of Antimicrobials and their use in Poultry Feeds. *Poult. Sci.*, **82**: 613–617.
30. Khovidhunkit, W., Kim, M., Memon, R. A., Shigenaga, J. K., Moser, A. H., Feinfold K. R. and Grunfeld, C. 2004. Thematic Review Series; the Pathogenesis of Atherosclerosis. Effects of Infection and Inflammation on Lipid and Lipoprotein Metabolism Mechanism. *J. Lipid Res.***45:**1169-1196
31. Lawless, J. 1995. *The Illustrated Encyclopedia of Essential Oils*. Element Books Ltd, Shaftesbury, UK, 256P.
32. Leeson, S. 1984. Growth and Carcass Characteristics of Broiler Chickens Fed Virginiamycin. *Nutr. Res.*, **29**: 1383-1389.
33. Mathivanan, R. and Kalaiarasi, K. 2007. *Panchagavya* and *Andrographis panculata* as Alternatives to Antibiotic Growth Promoters on Hematological, Serum Biochemical Parameters and Immune Status of Broilers. *Poult. Sci.*, **44**: 198-204.
34. Middleton, A. and Hui, K. P. 1982. Inhibition of Hepatic S-3-hydroxy-3-methylglutaryl-Coa Reductase and *In vivo* Rates of Lipogenesis by Mixture of Pure Cyclic Monoterpenes. *Biochem. Pharmacol*., **31**: 2897-2901.
35. Miles, R. D., Butcher, G. D., Henry P. R. and Littell, R. C. 2006. Effect of Antibiotic Growth Promoters on Broiler Performance, Intestinal Growth Parameters and Qualitative Morphology. *Poult. Sci*., **85**: 476–485.
36. Mohan, B., Kadiveral, R., Nnatarajan, A. and Bhaskaran, M. 1996. Effect of Probiotic Supplementation on Growth, Nitrogen Utilization and Serum Cholesterol in Broilers. *Br. Poult. Sci.*, **37**: 395-401.
37. National Research Council. 1994. *Nutrient Requirements of Poultry*. 9th Rev. Edition, National Academy Press, Washington, DC, 174 P.
38. Omidbaygi, R. 2004. *Medicinal Plants.* Tarbiat Modares University, Tehran, Iran, 348P.
39. Onibi, G. E., Scaife, J. R., Murray, I. and Fowler, V. R. 2000. Supplementary α-tocopherol Acetate in Full Fat Rape Seed-based Diets for Pigs: Influence on Tissue α -tocopherol Content, Fatty Acid Profile and Lipid Oxidation. *J. Sci. Food. Agric.*, **80**: 1625-1632.
40. Panda, A. K., Reddy, M. R., Rama Rao, S. V., Raju, M. V. L. and Praharaj, N. K. 2000. Growth, Carcass Characteristics, Immunocompetence and Response to *Escherichia coli* of Broilers Fed Diets with Various Levels of Probiotic. *Archiv für Geflügelkunde*, **64**: 152-156.
41. Patterson, J. A. and Burkholder, K. M. 2003. Application of Prebiotics and Probiotics in Poultry Production. *Poult. Sci.* **82**: 627–631.
42. Pelicano, E. R. L., de Souza, P. A., de Souza, H. B. A., Leonel, F. R., Zeola, N. M. B. L. and Boiago, M. M. 2004. Productive Traits of Broiler Chickens Fed Diets Containing Different Growth

Promoters. *Rev. Bras. Cienc. Avic.*, **6**: 177–182.
43. Pelicano, E. R. L., Souza, P. A., Souza, H. B. A., Figueiredo, D. F., Boiago, M. M., Carvalho, S. R. and Bordon, V. F. 2005. Intestinal Mucosa Development in Broiler Chickens Fed Natural Growth Promoters. *Rev Bras Cienc. Avic.,* **4**: 221- 229.
44. Rosen, G. D. 1995. Antibacterials in Poultry and Pig Nutrition. In: "*Biotechnology in Animal Feeds and Animal Feeding*", (Ed.): Wallace, R. J. and Chesson, A.. Wiley-VCH, Weinheim, Germany, PP. 143–172
45. Sakine, Y., Ebru, E., Reisli, Z. and Suzan, Y. 2006. Effect of Garlic Powder on the Performance, Egg Traits and Blood Parameters of Laying Hens. *J. Food. Sci.,* **86**: 1336-1339.
46. Santoso, U., Tanaka, K. and Ohtanis, S. 1995. Effect of Dried *Bacillus subtilis* Culture on Growth, Body Composition and Hepatic Lipogenic Enzyme Activity in Female Broiler Chicks. *Br. J. Nutr.* **74**: 523-529.
47. SAS Institute. 2003. *SAS User's Guide.* Version 9.1 Editon, SAS Inst. Inc., Cary, NC.
48. Shapiro, S. and Guggenheim, B. 1995. The Action of Thymol on Oral Bacteria. *Oral Microbiol. Immunol.*, **10**: 241-246.
49. Schleicher, A., Fritz, Z. and Kinal, S. 1998. Zastosowanie Wybranych ziół w Mieszankach Treciwych dla Kurczt Rzenych [The Use of Some Herbs in Concentrates for Broiler Chickens]. *Rocz. Nauk. Zootech*., **25(3):** 213-244. (in Polis)
50. Silva, E. N., Teixeira, A. S., Bertechini, A. G., Ferreira, C. L. and Ventura, B. G. 2000. *Cienciae Agrotecnologia***, 24**: 224-232.
51. Stiles, J. C., Sparks, W. and Ronzio, R. A. 1995. The Inhibition of *Candida albicans* by Oregano. *J. Appl. Nutr.*, **47**: 96-102.
52. Tekeli, A., Çelik, L., Kutlu, H. R. and Gorgulu, M. 2006. Effect of Dietary Supplemental Plant Extracts on Performance, Carcass Characteristics, Digestive System Development, Intestinal Microflora and Some Blood Parameters of Broiler Chicks. *Proceedings of 12th European Poultry Conference,* Sept. 10-14, Verona, Italy, PP: 307-308.
53. Tekeli, A., Kutlu, H. R., Celik, L., Var, I., Yurdakul, E. and Avcy, A. 2008. The Use of Propolis as an Alternative to Antibiotic Growth Promoters in Broiler Diets. *Proceedings of 23th Worlds Poultry Congress,* June 30 - July 4, Brisbane, Australia, PP: 482-482.
54. Viveros, A. A., Brenes, M., Pizarro, G. and Castanb, M. 1994. Effect of Enzyme Supplemention of a Diet Based on Barly, and Actoclave Apparent Digestibility, Growth Performance and Got Morphology of Broilers. *Anim. Feed. Sci. Technol*., **48**: 237-251.
55. Vukic Vramjes, M. and Wenk, C. 1995. Influence of Dietary Enzyme Complex on the Performance of Broilers Fed on Bites with and without Antibiotic Supplementation. *Br. Poult. Sci.*, **36**: 265-275.
56. Witkamp, M. 1963. Microbial Populations of Leaf Litter in Relation to Environmental Conditions and Decomposition. *Ecological Society America,* **44(2):** 370-377
57. Wegmann, T. and Smithies, O. 1966. A Simple Hemagglutination System Requiring Small Amounts of Red Cells and Antibodies. *Transfusion,* **6**: 67-75.
58. Yu, S. G., Hsu, J. C. and Chiou, P. W. S. 1998. Effects of β-glucanase Supplementation of Barley Diets on Growth Performance of Broilers. *Anim. Feed. Sci. Technol*., **70**: 353–361.
59. Zhang, A. W., Lee, B. D., Lee, S. K., Lee, K. W., An, G. H., Song, K. B. and Lee, C. H. 2005. Effects of Yeast (*Saccharomyces cerevisiae*) Cell Componets on Growth Performance, Meat Quality, and Ileal Mucosa Development of Broiler Chicks. *Poult. Sci.*, **84**: 1015-1021.

اثر سطوح بومادران (*Achillea millefolium* L.)، آنتی بیوتیک و پروبیوتیک بر جمعیت میکروبی، پاسخ ایمنی، لیپیدهای سرم و عملکرد جوجه‌های گوشتی

س. یخکشی، ش. رحیمی و ح. ر. همتی متین

چکیده

این مطالعه جهت بررسی اثرات گیاه داروئی بومادران (*Achillea millefolium* L.)، پروبیوتیک (پریمالاک) و ویرجینیامایسین بر روی خصوصیات دستگاه گوارش، لیپیدهای سرم، پاسخ ایمنی، جمعیت میکروبی و عملکرد جوجه‌های گوشتی اجرا شد. تعداد ۲۵۰ قطعه جوجه‌ی گوشتی یک روزه‌ی نر (راس ۳۰۸) به صورت تصادفی به ۵ تیمار، با ۵ تکرار و ۱۰ پرنده در هر یک اختصاص داده شدند. تیمارها شامل شاهد، ویرجینیامایسین (ppm ۱۵)، پریمالاک (۰/۱ ٪ در جیره) و دو سطح پودر بومادران (۱/۵ و ۳ ٪ در جیره) بودند. بالاترین و پایین‌ترین ضریب تبدیل خوراک (FCR) به ترتیب در تیمارهای شاهد و آنتی بیوتیک در سن ۴۲ روزگی مشاهده شد ($P<0.05$). به‌علاوه، بالاترین و پایین ترین افزایش وزن بدن(BWG) به ترتیب به وسیله تیمارهای آنتی بیوتیک و شاهد به‌دست آمد ($P<0.05$). در بین تیمارها، بازده لاشه تفاوتی نداشت ($P>0.05$). اوزان نسبی سینه و ران در بین همه تیمارها مشابه بود ($P>0.05$). وزن نسبی بورس فابریسیوس، طحال و پاسخ ایمنی اولیه (کل تیتر، IgG وIgM) علیه سلول‌های خونی قرمز گوسفند (SRBC) بین تیمارها تفاوت معنی‌داری نداشت ($P>0.05$). کلسترول سرم، تری گلیسرید و سطوح لیپوپروتئین‌ها با چگالی بالا و پایین (HDL و LDL) به شکل متفاوتی به وسیله تیمارها تحت تاثیر قرار گرفتند ($P<0.05$). پایین‌ترین پارامترهای ذکر شده با تیمار بومادران ۳ ٪ به‌دست آمدند ($P<0.05$). بالاترین و پایین‌ترین تیتر آنتی بادی (پاسخ ایمنی ثانویه) به ترتیب در تیمارهای بومادران (۳ ٪) و آنتی بیوتیک مشاهده شد ($P<0.05$). بالاترین باکتری‌های اسید لاکتیک در چینه‌دان، ایلئوم و سکوم به وسیله پریمالاک به‌دست آمدند ($P<0.05$). به‌علاوه آنتی‌بیوتیک و بومادران (۳٪) سبب کاهش معنی‌داری در تعداد کل باکتری‌های هوازی و کلی‌فرم‌ها در چینه‌دان، ایلئوم و سکوم شدند ($P<0.05$). نتایج آزمایش انجام شده نشان می‌دهد که استفاده از بومادران (۳٪) می تواند موجب کاهش سطوح لیپیدهای سرم و بهبود سیستم ایمنی جوجه‌های گوشتی شود. همچنین این عمل می‌تواند باعث کاهش باکتری‌های پاتوژن در سیستم گوارشی شود، که این امر می‌تواند به بهبود سلامت روده و خوب بودن طیور کمک کند. پیشنهاد می‌شود که از بومادران به عنوان یک جایگزین برای آنتی بیوتیک استفاده شود.