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Full Length Research Paper

Evaluation of yarrow (*Achillea millefolium*) as a natural growth promoter in comparison with a probiotic supplement on performance, humoral immunity and blood metabolites of broiler chicks

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This experiment was conducted to evaluate the potential of yarrow in comparison with probiotic as growth promoter on performance, immune responses, and some biochemical and hematological parameters in broiler chicks. One hundred and ninety two day-old mix broiler chicks (Ross 308) were randomly assigned to four treatment groups (control, probiotic 15 mg/kg Protoxin™, and yarrow powder of 5 and 10 g/kg of diet). Four floor pen replicates of 12 chicks were randomly allocated to each treatment. Body weight, feed intake, and feed conversion ratios were recorded at 14, 28 and 42 d. On day 42 blood samples were collected in heparinized and none-heparinized vials from the wing vein and hematological and biochemical parameters were determined, respectively. Albumin to globulin and heterophil to lymphocyte ratios were also calculated. Antibody titers against Newcastle and Influenza viruses and sheep red blood cell (SRBC) were measured at 24 and 28 d of age, respectively. Results showed that body weight of broilers significantly ($P<0.05$) increased in probiotic treatment and decreased in 10 g/kg yarrow. Feed intake of broilers not significantly tended to decrease in control but feed conversion was not affected by dietary treatments. Most of the carcass characteristics of broilers slaughtered at 42 d were not influenced by treatments but abdominal fat pad significantly reduced ($P<0.05$) in probiotic group compared to control birds. Serum triglyceride level in probiotic and dietary yarrow groups was significantly lower than control birds ($P<0.05$). Other blood parameters were not statistically affected by dietary treatments. No significant impact of additives was observed on humoral immune responses but H/L ratio decreased markedly in birds fed probiotic. The current results suggest that addition of yarrow powder seem not to have a positive influence on growth performance and failed to elevate immune responses thus, it could not be considered as a natural growth promoter for broiler chicks.

Key words: Broiler, yarrow, probiotic, immunity, serum biochemistry, blood hematology.

INTRODUCTION

Antibiotics at sub therapeutic doses have been widely used in animal feed as growth promoters to enhance animal growth performance and production. In the presence of low levels of antibiotics, resistant cells survive and grow producing an antibiotic resistant population in the final products. Therefore, the application of antibiotics as growth promoters (AGP) in the animal feed has been limited in the European Union since January 2006. Consequently, probiotics and prebiotics, herbs, spices and various plant extracts have received increased attention as possible antibiotic growth promoter

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substitutions. Beneficial effects of bioactive plant substances in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune responses and antibacterial, antiviral and antioxidant actions. Probiotics mean in favor of life, exactly the opposite to antibiotics that is against life. Probiotics are defined as live microbial agents that can beneficially influence the intestinal microbial balance of host. Probiotics supplement are composed of single or multiple species of bacteria, fungi and yeasts, which can survive or establish in intestine of host and improve the characteristics of intestinal micro-flora (Fuller, 1989). The inclusion of probiotics in livestock feed is designed to encourage the growth of certain strains of bacteria in the gut at the expense of less desirable ones. Their effect on production reflects in reduction of risk of diseases, improvement of the immune system function and exhibit significant influence on morpho-functional characteristics of intestines (Yang et al., 2009). These effects lead to improvement of growth rate, feed efficiency and reduced mortality in broiler chicks.

Yarrow (*Achillea millefolium*), belonging to the Asteraceae family, is used widely in many parts of the world. Many compounds have been isolated from *Achillea* species, including flavonoids, sesquiterpene lactones and polyacetylenes and for many centuries various species of the genus have been used as folk medicines for curing of various diseases (Saeidnia et al., 2005). Yarrow has been reported to be used as a medicine for disorders of the respiratory, digestive, hepatobiliary, cardiovascular, urinary, and reproductive systems (Blumenthal et al., 1998). Extracts of yarrow have demonstrated antimicrobial activity against a wide range of bacteria including *Streptococcus pneumoniae, Clostridium perfringens, Candida albicans, Mycobacterium smegmatis, Acinetobacter lwoffii and Candida krusei* also antioxidant activity (Candan et al., 2003; Stojanovic et al., 2005). Displaying antimicrobial and antioxidant properties, yarrow was supposed to have the potential to be applied as AGP in broilers diet. Thus, the current study was designed to evaluate the possibility of applying different levels of yarrow compared to a probiotic supplement (Protoxin™) on performance, some immune responses, biochemical, and hematological blood parameters of broiler chicks.

MATERIALS AND METHODS

Animals and diets

One hundred and ninety two day-old mix broiler chicks (Ross 308) were purchased from a local hatchery. On arrival, birds were weighed and randomly assigned to one of four treatments with four replicates of 12 birds based on a completely randomized design. The dietary treatments consisted of the basal diet as control, 5 and 10 g/kg yarrow, 15 mg/kg Protoxin™ (as probiotic) added to the basal diet in inclusion of corn. The basal diet was formulated (UFFDA) to meet or exceed the nutrient requirements of broilers provided by Ross broiler catalogue (2007) according to Table 1. Chicks were raised on floor pens (120 × 120 × 80 cm) for 6 weeks and had free access to food and water throughout the experimental period (0 to 42 d). The lighting program consisted of a period of 23 h light and 1 h of darkness throughout the experimental period. The ambient temperature was initially set at 33°C and gradually decreased by 3°C per week to 24°C on the third week and was then kept constant.

Data collection

Chicks were individually weighed upon arrival and after the initiation of the experiment body weight was recorded on a pen basis at 14, 28, and 42 days of age after a 12 h feed withdrawal. Feed intake was measured in groups in similar periods, individual feed consumption was calculated with regard to bird mortality during the experiment; consequently, feed conversion ratio (feed intake/weight gain) was calculated. At 42 days of age and after overnight fasting, two birds per replicate randomly chosen were slaughtered and abdominal fat, liver, pancreas, gizzard, heart, cecum, and small intestine were collected, weighed, and calculated as a percentage of live body weight. The length of small intestine was also measured and recorded. All birds were intra-muscularly vaccinated against Influenza and Newcastle viruses at 14 d of age. At 24 d of age, two birds per replicate were randomly chosen and blood samples were collected from brachial vein and centrifuged at 3000 rpm for 10 min to obtain serum (SIGMA 4-15 Lab Centrifuge, Germany). Antibody titers against Newcastle and Influenza viruses were measured using Hemagglutination Inhibition Test. At 22 d of age, 12 birds from each treatment groups were injected i.v. with 1 ml of a 1% suspension of sheep red blood cell (SRBC) prepared in phosphate-buffered saline. Blood samples were collected from challenged birds 6 d after, anti-SRBC titers were measured by the microtiter procedure of Wegmann and Smithies (1966). All titers were expressed as the $\log_2$ of the reciprocal of the highest dilution giving visible hemagglutination. Blood samples were collected in non-heparinised tube at 42 d of age from two birds in each replicate by puncturing the brachial vein and the blood was centrifuged to obtain serum. Individual serum samples were analyzed for total protein, albumin, total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) cholesterol and triglyceride (Kit package, Pars Azmoon Company; Tehran, Iran). Globulin concentration in serum was computed by subtracting albumin concentration from proteins, consequently albumin to globulin ratio was calculated.

At 42 d of age, two birds per replicate were selected and blood samples were collected by syringes containing heparin to avoid blood clot formation. Blood smears were prepared using May-Greenwald-Giemsa stain. One hundred leukocytes per samples were counted by heterophil to lymphocyte separation under an optical microscope (100 x oil immersion) then heterophil to lymphocyte ratio was calculated and recorded (Gross and Siegel, 1983). Also, the red blood cell (RBC) and white blood cell (WBC) counts were determined by a hemocytometer method using Natt-Herrick solution; hematocrit (HCT) and hemoglobin (HGB) values were measured by microhematocrit and cyanmethemoglobin methods respectively (Kececi et al., 1998).

Statistical analysis

The obtained data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS Institute (2008). Means were compared using Duncan multiple test. Statements of statistical significance are based on $P<0.05$.

Table 1. Ingredients and composition of the basal diet.

Diet composition (g/kg)	Starter (1 to 14 d)	Grower (14 to 28 d)	Finisher (28 to 42 d)
Corn (analyzed CP 8%)	545.5	540	567
Soybean meal (analyzed CP 43%)	401	390	360
Soybean oil	11	33.2	39
Calcium carbonate (38% calcium)	10.6	8.9	8.7
Dicalcium phosphate	19.1	17.3	15.7
DL-Methionine	3	2.1	1.6
L-lysine Hydrochoride	1.3	-	-
Vitamin premix[1]	2.5	2.5	2.5
Mineral premix[2]	2.5	2.5	2.5
Salt	3.5	3.5	3
Total	1000	1000	1000
Calculated chemical composition			
Metabolizable energy (MJ/kg)	11.76	12.47	12.76
Crude protein (g/kg)	215	210	200
Ca (g/kg)	9.7	8.6	8.1
Available phosphorous (g/kg)	4.6	4.3	4
Methionine + Cystine (g/kg)	10	9	8.2
Lysine (g/kg)	13.2	11.9	11.1

Dicalcium phosphate contained: 16% phosphorous and 23% calcium. 1- Vitamin premix per kg of diet: Vitamin A (retinol), 2.7 mg; Vitamin D3 (Cholecalciferol), 0.05 mg; Vitamin E (tocopheryl acetate), 18 mg; Vitamin k3, 2 mg; thiamine 1.8 mg; riboflavin, 6.6 mg; panthothenic acid, 10 mg; pyridoxine, 3 mg; cyanocobalamin, 0.015 mg; niacin, 30 mg; biotin, 0.1 mg; folic acid, 1 mg; choline chloride, 250 mg; Antioxidant 100 mg. 2- Mineral premix per kg of diet: Fe ($FeSO_4.7H_2O$, 20.09% Fe), 50 mg; Mn ($MnSO_4.H_2O$, 32.49% Mn), 100 mg; Zn (ZnO, 80.35% Zn), 100 mg; Cu ($CuSO_4.5H_2O$), 10 mg; I (KI, 58% I), 1mg; Se ($NaSeO_3$, 45.56% Se) , 0.2 mg.

RESULTS AND DISCUSSION

Performance indices and carcass characteristics

Table 2 summarizes the impact of dietary treatments on productive parameters of chicks at different periods. Probiotic supplementation significantly ($P<0.05$) increased body weight at different periods compared to yarrow and control birds, this increase at 42 d was more pronounced. Control birds tended to have a lower feed intake over the entire experimental periods although the differences did not reach statistical significance. The additives did not markedly ($P>0.05$) influence feed conversion ratios of chicks; nevertheless the most efficient feed conversion throughout the study was observed in chicks fed the probiotic supplemented diets. The lowest body weight observed in chicks receiving 10 g/kg yarrow could partially be due to the presence of some anti-nutritional factors in yarrow such as tannins, hydrocyanic acid, and linalool. Tannins can bind proteins in digestive tracts and reduce protein absorption; also, hydrocyanic acid has the potential to prohibit tissue respiration by preventing the activity of cytochrome oxidase enzyme thus resulting in decreased growth performance. In line with our results, Rose et al. (2009) showed that feeding yarrow to broiler chicks did not result in any significant improvement of productive traits and birds fed diets containing no yarrow tended to have higher feed intakes and growth rates. This is while Cross et al. (2007) who investigated the effect of the dietary inclusion of 5 culinary herbs (thyme, oregano, marjoram, rosemary or yarrow) or their essential oils on the growth performance and nutrient digestibility in broiler chicks reported that dietary thyme oil or yarrow herb inclusion had positive effects on chick performance. These researchers not only did not observed any marked increase in body weight of broiler chicks fed diets supplemented with yarrow oil but also noted a significant increase in feed conversion ration due to dietary supplementation with yarrow oil.

Probiotics on one hand suppresses the growth of pathogenic microorganisms in the intestine and incidence of diarrhea, on the other hand have the potential to increase the bioavailability of dietary minerals resulting in an improved growth rate and feed efficiency. In addition, it has been demonstrated that probiotics inhibit the *in vitro* growth of many enteric pathogens (Choudhari et al., 2008). In accord to our results, Panda et al. (2005) reported that application of probiotics in broilers diet increased final body weight gain and improved FCR at week 6 of age. As Table 3 displays, carcass yield of broiler was not statistically influenced by dietary treatments. Feeding probiotic supplemented diets significantly decreased abdominal fat pad of birds

Table 2. Effect of experimental diets on performance of broilers at different ages.

Performance parameters	Dietary treatments				
	Control	5 g/kg yarrow	10 g/kg yarrow	15 mg/kg protoxin	SEM
Body weight (g)					
14 d	275^{b}	247^{c}	291^{ab}	299^{a}	10.25
28 d	946^{b}	929^{b}	960^{ab}	1019^{a}	29.09
42 d	1956^{b}	1951^{b}	1904^{bc}	2138^{a}	57.09
Daily feed intake (g/d)					
0-14 d	28.9	28.1	29.4	31.5	1.99
14-28 d	75.9	78.9	76.7	76.8	2.76
28-42 d	174.18	184.4	175.3	181.1	5.65
0-42 d	90.88	92.7	91.3	95.6	2.21
Feed: gain (g:g)					
0-14 d	1.78	1.77	1.76	1.75	0.0111
14-28 d	1.46	1.51	1.52	1.49	0.0320
28-42 d	2.37	2.47	2.32	2.27	0.051
0-42 d	1.95	2.06	2.06	1.88	0.089

Values in the same row not sharing a common superscript differ significantly (P <0.05).

Table 3. Effect of experimental diets on carcass yield, abdominal fat and internal organ weight of broilers at d 42.

Carcass traits	Dietary treatments				
	Control	5 g/kg yarrow	10 g/kg yarrow	15 mg/kg protoxin	SEM
Carcass yield*	73.8	73.8	73.5	74.8	0.69
Abdominal fat*	1.76^{a}	1.67^{ab}	1.72^{ab}	1.36^{b}	0.18
Liver*	2.17	2.26	2.36	2.15	0.14
Gizzard*	2.05	2.42	2.38	2.13	0.16
Heart*	0.591	0.569	0.601	0.585	0.048
Pancreas*	0.228	0.268	0.267	0.238	0.021
Intestine*	3.22	3.71	3.67	3.23	0.18
Cecum*	0.618	0.742	0.754	0.614	0.07
Intestine length**	173	179	166	173	7.14
Cecum length**	38.3	38.1	38	39.1	1.85

Values in the same row not sharing a common superscript differ significantly (P <0.05). *Percentage of live body weight. **cm.

compared to broiler chicks in control group (P<0.05). Nevertheless, the relative weights of internal organs of chicks slaughtered at 42 d were not affected by treatments (Table 3). Previous studies have demonstrated the positive impact of probiotics feeding on abdominal fat content of broilers (Pelicano et al., 2003). This result may be attributed to the reducing effect of probiotics on fat deposition (Mohan et al., 1996). Similar to our observation on carcass yield Pelicano et al. (2003) also stated that presence or absence of probiotics had no effect on carcass yield but the authors noted that leg yield was higher in the birds that received probiotics.

Immune responses

The impact of additives on immune related parameters is listed in Table 4. Antibody titers against Newcastle and Influenza viruses were not significantly affected by the treatments. However, control birds showed a slightly higher response to SRBC immunization compared to other treatments (P>0.05). It has been demonstrated that humoral immunity is a viable part of the immune system (Scott, 2004) and as yarrow has been reported to have antimicrobial and antioxidant properties (Candan et al., 2003; Stojanovic et al., 2005) humoral immune

Table 4. Effect of experimental diets on antibody titers against Newcastle and Influenza at d 24 and SRBS at d 28, heterophil to lymphocyte and albumin to globulin ratios at d 42.

Variables	Dietary treatments				
	Control	5 g/kg yarrow	10 g/kg yarrow	15 mg/kg protoxin	SEM
Newcastle (log_2)	5.62	4.12	5.75	4.62	0.6
Influenza (log_2)	3.62	4.38	4.62	3.75	0.58
SRBC (log_2)	6	5	4.87	4.37	0.89
H/L	0.36^{ab}	0.37^{ab}	0.49^{a}	0.25^{b}	0.054
A/G	0.85	0.54	0.56	0.60	0.21

Values in the same row not sharing a common superscript differ significantly (P <0.05).

parameters were expected to be elevated by feeding yarrow but they were not. It is likely that a higher dosage of yarrow herb may be needed to stimulate humoral immune response to ND and AI vaccines. In addition, the lack of effect of the yarrow on immune responses may relate to the composition of the basal diet (Table 1) Ross broiler catalogue (2007) and/or the environmental conditions. The diet contained highly digestible ingredients so that bacterial growth in the intestine probably may have been limited. Antimicrobial agents may have more impact when the diet used is less digestible. In addition, it is known that well-nourished, healthy chicks do not respond to antibiotic supplements provided that they are housed under clean and disinfected conditions (Lee et al., 2003). Unfortunately, there is no report in the literature concerning the impact of yarrow on antibody production in broilers but alike our observation Toghyani et al. (2010a) also failed to observe any significant impact of thyme medicinal herb on antibody production against ND and AI viruses. Previous work with chickens has demonstrated that probiotics enhance the systemic antibody response to soluble antigens, such as trinitrophenyl (TNP)-keyhole limpet hemocyanin (KLH) and KLH alone, which, like SRBC, are classified as hymus-dependent immunogens (Huang et al., 2004; Koenen et al., 2004). However, we failed to monitor any positive impact of Protoxin™ on antibody production titer in serum of chicks at different ages.

In accord to our results, Haghighi et al. (2005) reported that antibody response to soluble antigens in chickens might not be enhanced feeding diets supplemented by probiotics. These scientists concluded that the immunomodulatory activities of probiotics in enhancing the antibody response are highly dependent on the antigen, immunization regimen, type, and number of species of bacteria present in probiotics, and genetic background of the host. In fact, there have been reports that combining probiotics with immunization may not enhance specific antibodies and could even result in the reduction of the antibody response in serum (Balevi et al., 2001; Dalloul et al., 2003); this could be seen in SRBC response of probiotic fed birds compared to control birds. Birds receiving probiotics had the lowest H/L ratio (P<0.05) while, A/G ratio did not significantly differ among dietary treatments although it tended to be higher in control birds since globulin concentration was slightly lower in control chicks but the differences observed did not reach statistical significance (Table 4). A/G ratio has been used as an indicator of immune responses so that in 1986 Griminger stated that high globulin level and low A/G ratio signify better disease resistance and immune responses. The reliability of H/L Ratio as a biological index of stress in avian species is also, well documented (Maxwell, 1993; Bedanova et al., 2007). The lower H/L ratio observed in probiotic group implies the positive influence of Protoxin™ on reducing stress in broilers.

Blood biochemical and hematological parameters

According to Table 5 only triglyceride concentration of serum was significantly (P<0.05) influenced by the treatments so that the control birds had the highest value compared to other treatments. In addition, hematocrit percentage was recorded to be slightly higher in 5 g/kg yarrow and probiotic treatments. Other parameters tested did not vary statistically among dietary treatments. Some herbs have been reported to possess hypolipidemic and hypocholesteremic properties such as black seed (AL-Beitawi and El-Ghousein, 2008) and garlic (Kim et al., 2009) in broilers and *E. koreanum* in egg yolk in layers (Park et al., 2010). In our research hypolipidemic effect of yarrow and Protoxin was obvious and reflected in lower triglyceride concentration but no hypocholesteremic effects of these additives was observed, probably because the absence or presence of cholesterolaemic effects of dietary components in an animal depend on various factors such as breed, gender and age, and also on the composition of the feed. Although, HDL-cholesterol concentration none significantly elevated by feeding 10 g/kg yarrow compared to control and probiotic groups. Unfortunately, there are no reports available on the effectiveness of yarrow or probiotics on the biochemistry of serum and the possible mechanisms. Blood serum protein is a labile biochemical system, precisely reflecting the condition of the organism and the changes happening to it under influence of internal and external factors. High serum protein levels have been

Table 5. Effect of experimental diets on blood biochemical and hematological parameters of broilers at d 42.

Blood parameters	Dietary treatments				
	Control	5 g/kg yarrow	10 g/kg yarrow	15 mg/kg Protoxin	SEM
Protein*	3.34	3.65	3.4	3.48	0.34
Albumin*	1.2	1.2	1.1	1.2	0.33
Globulin*	2.1	2.42	2.26	2.26	0.33
Triglyceride*	142[a]	98[b]	112[b]	111[b]	13.55
Total cholesterol*	112	106	118	108	12.61
HDL-cholesterol*	71	64	73	80	7.23
LDL-cholesterol*	32	31	33	32	3.64
RBC ($\times 10^6/\mu l$)	2.58	2.44	2.58	2.5	0.16
WBC ($\times 10^3/\mu l$)	17.05	16.63	19.69	16.74	5.93
Haemoglobin*	10.6	11.4	10.2	10.7	0.49
Haematocrit (%)	30	32	30	32	1.3

Values in the same row not sharing a common superscript differ significantly ($P < 0.05$). *(mg/100 ml).

reported to be an indicative of osmoregulatory dysfunction, hemodilution, or tissue damage surrounding blood vessels (Hille, 1982) or it could be attributed to an increase in the immunoglobulin level and total globulin concentration (Hussein, 1996) which were not statistically evident in the present experiment. Changes in the physiological state often reflect alteration of hematological values hence, hematological parameters have often been associated with health indices and are of diagnostic significance in routine clinical evaluation of the state of health.

Clinical chemical analysis is a fundamental tool used to diagnose and predict the outcome of diseases and to monitor the effects of therapeutic, nutritional, and environmental management in human and veterinary medicine. For instance, Toghyani et al. (2010b) indicated the favorable influence of black seed on hematopoiesis of broiler chicks. However, as Table 5 displays the additives applied as growth promoters did not significantly influence hematological parameters tested in this study.

Conclusion

The overall results of the current study showed that yarrow powder seems not to have the potential to positively influence growth performance and immune responses of broiler chicks thus; it could not be considered as a natural growth promoter. While, probiotic supplementation improved performance indices and carcass quality by reducing abdominal fat content.

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