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PAPER

Effect of different dietary levels of rosemary (*Rosmarinus officinalis*) and yarrow (*Achillea millefolium*) on the growth performance, carcass traits and ileal microbiota of broilers

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Abstract

The effect of increasing dietary levels of rosemary and yarrow herb powders on the growth performance, carcass traits and ileal microbiota of broilers was studied. Three hundred and thirty-six one-day-old Ross 308 male chickens were allocated to one of the following treatments (six replicates of 8 birds per treatment): control (basal diet with no rosemary or yarrow herbs added), and basal diet with the addition of either 0.5, 1.0 or 1.5% of rosemary herb, or 0.5, 1.0, or 1.5% of yarrow herb. Significant differences were observed ($P<0.05$) between treatments in the starting (d 1-21) and growing (d 22-42) periods, and in the average feed intake in the starting period. When the whole experimental period was considered, the best-feed conversion rates ($P<0.05$) were achieved for the highest rosemary and yarrow supplementation levels. Yarrow supplementation resulted in better-feed conversion rates ($P<0.05$) than the control treatment, for all the periods. No significant differences ($P>0.05$) were found in the final body weight or in most of the carcass traits. The gastrointestinal tract weight, relative to body weight, increased ($P<0.05$) due to rosemary and yarrow supplementation, compared with the control treatment. At 42 days of age, the rosemary supplementation increased the *Lactobacilli* counts and decreased the *Escherichia coli* counts more than the control and yarrow supplemented treatments. In conclusion, under the conditions of the present work, yarrow supplementation mainly improved growth performance, while rosemary supplementation showed the best effects on ileal microbiota, both compared with the control treatment.

Introduction

The ban of growth-promoting antimicrobials in some countries, due to the risk of antibiotic-resistant bacteria in humans and increasing concern by consumers about food safety, has led to the search for non-therapeutic alternatives, including enzymes, organic acids, probiotics, prebiotics and phytogenic feed additives, that are able to support the productive performance and prevent the incidence of some diseases in poultry (Huyghebaert *et al*., 2011). Among such alternatives, phytogenic feed additives (phytobiotics or botanicals) have received increasing attention in recent years (Christaki *et al*., 2012; Vidanarachchi *et al*., 2005). Windisch *et al*. (2008) classified phytogenic feed additives as herbs (leaves, flowering, non-woody, and non-persistent plants), spices (herbs with an intensive smell or taste), essential oils (volatile lipophilic phytochemicals derived by cold expression or by steam or alcohol distillation methods), or oleoresins (derived by non-aqueous solvent extraction). Phytogenic feed additives above all contain terpenes and phenolic compounds (Brenes and Roura, 2010), which are thought to be the main compounds responsible for their pharmaceutical properties (Christaki *et al*., 2012).

The research carried out so far suggests that phytogenic feed additives could have broad a wide range of properties that could improve the growth performance and health of poultry,by stimulating the feed intake and antimicrobial, coccidiostatic and antioxidant effects (Brenes and Roura, 2010; Hippenstiel *et al*., 2011; Wallace *et al*., 2010). Phytogenic feed additives are considered as GRAS (Generally Recognized As Safe). However, due to the vast variety of potentially active substances in phytogenic feed additives, safety concerns cannot be excluded (Wallace *et al*., 2010; Hashemi and Davoodi, 2011). Conversely, it can be assumed that the effect of phytogenic feed additives on performance parameters will largely depend on their inclusion level in the feed (Applegate *et al*., 2010). Therefore, the knowledge of their optimum dietary concentration should be established.

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Rosemary (*Rosmarinus officinalis*) and yarrow (*Achillea millefolium*) have been used throughout history as herbal remedies for numerous human afflictions (Al-Sereiti *et al*., 1999; Benedek and Kopp, 2007). These plants contain several terpenes and phenolic compounds in different proportions (Bimbirait *et al*., 2008; Mathlouthi *et al*., 2012; Vèrma *et al*., 2011; Wojdyło *et al*., 2007). However, except for the work of Cross *et al*. (2007), little research has been conducted on comparing the effectiveness of rosemary and yarrow as growth promoters in broilers, and literature comparing the effects of increasing amounts of these plants in broiler diets is lacking. The aim of the present work was to determine the effect of increasing dietary levels of rosemary and yarrow on the growth performance, carcass traits and ileal microbiota of broilers.

Materials and methods

Animals, housing, and general management

The use and care of the birds and procedures in this study were approved by the Islamic Azad University Ethics Committee. Three hundred and thirty-six one-day-old male chickens of the Ross 308 strain (Aviagen, Newbridge, UK) were purchased from a commercial hatchery. The broiler chicks were placed in 1.25×1.25 m cages (0.20 m^2 per bird), whose floor was covered with shredded paper. Each cage was equipped with a pan feeder and a manual drinker. The research facility was an open-sided poultry barn with thermostatically controlled curtains, equipped with thermostatically controlled gasoline rocket heaters, overhead sprinklers, wall-mounted fans at both ends of the barn, and fluorescent tubes in the ceiling fixtures. The facilities were cleaned and disinfected before the beginning of the experiment. Ambient temperature was set at 32°C at placement and then decreased gradually to achieve 24°C from week 3 onwards. Lighting was constant on day 1. The light regime was 23L:1D from day 2 till the end of the study. The birds were vaccinated against infectious bronchitis disease (1st and 7th day of age), Newcastle disease (1st and 7th day of age), influenza disease (1st day of age) and Gumboro disease (21st day of age). All vaccines were provided by Razi Co. (Tehran, Iran). Feed (mash form) and water were provided *ad libitum* throughout the whole trial.

Experimental design and diets

The chicks were assigned to one of the following treatments: control (basal diet with no added rosemary or yarrow herbs), and the same basal diet with either 0.5, 1.0 or 1.5% of rosemary herb, or 0.5, 1.0 or 1.5% of yarrow herb added. Each treatment had six replicates, thus a total of 42 groups was obtained with 8 birds each. A standard commercial feeding programme was adopted and it consisted of a starter diet, until the chicks were 21 days old, followed by a grower diet up to the end of the experiment at 42 days of age. Both feeds were maize-soybean meal based and did not contain any antibiotic feed additives (Table 1). The rosemary and yarrow herbs (powdered form) were purchased from a local pharmacy store and their chemical composition was determined according to AOAC (1990).

Measurements, sample collection, and microbial enumeration

The body weights of the chicks and feed consumption were weekly determined by replicate. The body weight gain (BWG, g/d), feed intake (FI, g/d), and feed conversion ratio (FCR, feed-to-gain g/g) within each treatment were determined by period and globally. At the age of 42 days, after 4 hours of fasting to obtain complete evacuation of the gut, six chickens per treatment (one from each replicate) that had weights closest to the mean weight of the cage were selected and euthanized to determine the carcass traits and organ weights. The birds were plucked, and the feet, head, and wingtips were removed; they were then eviscerated before determining the carcass weight. The weights of the breasts, drumsticks, wings, liver and bile, and the whole gastrointestinal tract were recorded.

Six chickens per treatment (one from each replicate) were selected, as explained above, and euthanized in order to measure the microbial population at at 21 and 42 days of age. The ileum from each euthanized bird was quickly dissected and the digesta contents were collected in sterilized sampling tubes and immediately transferred to the laboratory. Ten-fold serial dilutions of 1 g of sample were made from these contents in a phosphate buffer solution (10^{-1}-10^{-6}). Subsequently, 100 μL was removed from 10^{-4}, 10^{-5}, and 10^{-6} dilutions and poured onto petri dishes containing the culture media. *Lactobacilli* were cultured in De Man, Rogosa and Sharpe agar and incubated at 37°C in anaerobic conditions for 72 h. *Escherichia coli* were cultured in eosin methylene blue agar and incubated at 37°C under aerobic conditions for 48 h. The bacterial colony forming units (CFU) in the petri dishes were counted using a colony counter. The counts were reported as log10 CFU per one g of sample.

Statistical analyses

Data were analysed using the GLM procedure of SAS 8 (SAS Institute, Inc., 2000). The statistical design was Y_{ij} = + T_j + e_{ij}, where Y_{ij} is the dependent variable; represents the overall mean; T_j is the effect of the treatment; e_{ij} is the residual error. Least squares means were compared using Tukey's test. The linear and quadratic responses to supplementation levels within the rosemary and yarrow treatments were investigated using appropriate contrast coefficients. The responses to herb supplementation were investigated through preplanned orthogonal contrasts (control *vs.* both rosemary and yarrow supplemented treatments, and rosemary *vs.* yarrow treatments). Statistical significance was declared at $P<0.05$.

Table 1. Experimental diets fed to broiler chickens.

	Starter	Grower
Ingredients, g/kg		
Maize	514.6	562.0
Soybean meal (44%)	393.5	316.5
Soybean oil	31.8	66.0
Dicalcium phosphate	21.1	17.3
Wheat bran	15.0	15.0
Calcium carbonate	9.6	8.6
Salt	2.7	2.8
Sodium bicarbonate	2.8	2.7
Vitamin mixture[°]	2.5	2.5
Mineral mixture[#]	2.5	2.5
DL-methionine	2.4	2.6
L-lysine HCL	0.8	0.6
L-threonine	0.7	0.9
Calculated analysis[§]		
Metabolizable energy, MJ/kg	12.20	13.41
Crude protein, %	22.20	19.20
Lysine, %	1.27	1.05
Threonine, %	0.90	0.80
Methionine, %	0.58	0.56
Tryptophan, %	0.33	0.27
Calcium, %	0.96	0.81
Available phosphorus, %	0.54	0.46

°Contents per kilogram: vitamin A, 3,600,000 U; vitamin D3, 800,000 U; vitamin E, 7200 U; vitamin K_3, 800 mg; thiamine, 720 mg; riboflavin, 2640 mg; calcium pantothenate, 4000 mg; niacin, 12,000 mg; pyridoxine, 1200 mg; folic acid, 400 mg; vitamin B_{12}, 6 mg; biotin, 40 mg; choline, 100,000 mg. #Contents per kilogram: Mn, 39680 mg; Fe, 20000 mg; Zn, 33880 mg; Cu, 4000 mg; I, 400 mg; Se, 80 mg. §According to National Research Council (1994).

Results and discussion

Growth performance

The effects of dietary supplementation with rosemary and yarrow herb powders on growth performance are presented in Table 2. As far as the rosemary supplementation level is concerned, FI and ADG showed quadratic responses (P<0.05) in the starting period, both being lower in the R1.0 treatment than in the R0.5 one (P<0.05). As a result, FCR was numerically higher in the R1.0 treatment than in the R0.5 and R1.5 treatments, and its response tended to be quadratic (P=0.108). During the growing period, FI showed a quadratic response (P<0.01) to the rosemary supplementation level, the numerically highest value being observed in the R1.0 treatment, but no differences (P>0.05) were observed between treatments in FI, in BWG or in FCR. When the whole experimental period was considered, no effects (P>0.05) on ADG or FCR were observed, due to the rosemary supplementation level, but FI showed a quadratic response (P<0.05), with the numerically highest value in the R1.0 treatment. The yarrow supplementation level during the starting period led to a quadratic response in FI (P<0.001), which showed the highest value (P<0.05) in the Y1.0 treatment, and a positive linear response in BWG (P<0.05). As a result, FCR showed a quadratic response (P<0.05), its value being higher (P<0.05) in the Y1.0 treatment than in the Y1.5 treatment. During the growing period, the yarrow supplementation level did not affect (P>0.05) FI, whereas ADG and FCR showed a positive and a negative linear response (P<0.05), respectively. When the whole experimental period was considered, no differences (P<0.05) between treatments were observed in FI or ADG, due to the yarrow supplementation level, while the lowest (P<0.05) FCR was observed in the Y1.5 treatment. However, the Rosemary supplementation improved (P<0.05) FCR in the starting and whole experimental periods, compared with the control treatment, while yarrow supplementation resulted in a better (P<0.01) FCR for all the periods. No differences (P>0.05) between Rosemary and yarrow supplementation were observed in any of the studied parameters.

Cross *et al*. (2007) found that including 1% yarrow herb in the diet resulted in a better growth performance of broilers at 28 days of age than supplementation with the same amount of rosemary herb, but 0.1% of rosemary oil was better than the same amount of yarrow oil supplementation. However, unlike the present work, those authors found none of the supplemented treatments better than the control. Our results differ from those of Yakhkeshi *et al*. (2012), who observed that 1 and 3% yarrow herb supplementation in the diet did not improve the growth performance of broilers more than the control treatment during the starting and growing periods or over the whole experimental period, but the results obtained with 0.5% supplementation were better than those obtained with the control treatment in all the periods. Moreover, Sharifi *et al*. (2013) observed that 0.2% yarrow herb supplementation had a negative effect on the growth performance of broilers during the growing and finishing periods and in the overall experimental period, compared with the control treatment. On the other hand, Al-Kassie (2008) reported that 0.5 and 1% rosemary herb supplementation in the diet clearly improved broiler growth performance at 42 days of age, compared with the control treatment. Ghazalah and Ali (2008) also found that 0.5% rosemary herb supplementation in the diet gave better results than the control treatment at 49 days of age.

Literature shows contradictory results regarding the effects of other phytogenic feed additives on broiler growth performance. For instance, neither Erdogan *et al*. (2010) nor Jang *et al*. (2007) found any advantage in feeding diets with the addition of commercial mixtures of essential oils. On the contrary, Khattak *et al*. (2014), Giannenas *et al*. (2014) and Mathlouthi *et al*. (2012) found improvements in broiler growth when the bird diets were supplemented with commercial mixtures of essential oils, rosemary or oregano oil, respectively. However, Marzoni *et al*. (2014) did not find any effect on either the growth or slaughtering performance of Muscovy ducks fed a diet supplemented with rosemary leaves or orange peel extract. The discrepancies mentioned above could be due, at least in part, to the lack of homogeneity in the products tested by different authors, although they came from the same plants. In other words, the proportions of flowers, leaves and stems could have been different in the tested products or the processing

Table 2. Feed intake, average daily gain, and feed conversion rate of broilers fed diets containing either no plant feed additives, or 0.5%, 1.0% or 1.5% of rosemary or yarrow herb powders.

	Treatments										
	C	R			Y			SEM	P		
		R0.5	R1.0	R1.5	Y0.5	Y1.0	Y1.5		CxR	CxY	RxY
Starter period (1-21 d)											
FI, g/d	52.84^{a}	51.47^{ab}	46.99^{cd}	48.88^{bcd}	46.42^{d}	53.51^{a}	49.32^{bc}	0.398	<0.01	<0.01	ns
ADG, g/d	38.38^{ab}	39.73^{a}	35.18^{b}	37.54^{ab}	36.34^{ab}	38.80^{ab}	38.88^{ab}	0.371	ns	ns	ns
FCR, g/g	1.40^{a}	1.30^{bc}	1.34^{ab}	1.30^{bc}	1.28^{bc}	1.37^{ab}	1.24^{c}	0.010	<0.01	<0.01	ns
Grower period (22-42 d)											
FI, g/d	150.51	141.04	152.70	144.71	150.63	150.72	148.47	1.279	ns	ns	ns
ADG, g/d	81.10^{ab}	77.51^{b}	85.58^{ab}	84.46^{ab}	84.20^{ab}	84.93^{ab}	90.18^{a}	1.044	ns	0.09	0.08
FCR, g/g	1.86^{a}	1.83^{a}	1.78^{ab}	1.74^{ab}	1.78^{ab}	1.78^{ab}	1.63^{b}	0.015	ns	<0.01	0.08
Whole period (1-42 d)											
FI, g/d	101.68	96.28	99.87	96.82	98.53	102.12	98.89	0.671	<0.05	ns	ns
ADG, g/d	59.74	58.61	59.98	60.59	59.89	61.47	64.12	0.528	ns	ns	0.07
FCR, g/g	1.71^{a}	1.64^{ab}	1.67^{ab}	1.61^{bc}	1.63^{ab}	1.68^{ab}	1.52^{c}	0.011	<0.05	<0.01	ns

C, control; R, rosemary; Y, yarrow; FI, feed intake; ADG, average daily gain; FCR, feed conversion rate. $^{a-c}$In a row, the least squares means with a different superscript differ significantly (P<0.05); ns, not technique could have modified the active substances within the final product (Windisch *et al*., 2008; Hippenstiel *et al*., 2011). Another cause of the discrepancy could have been a different dosage of the active compounds in the final diet (Applegate *et al*., 2010). The composition of rosemary and yarrow herb powders used in the present work was (% air-dried basis): Dry matter, 88.8 and 86.1, crude protein, 10.1 and 11.8, ash, 14.0 and 8.0, crude fat, 3.0 and 2.0, and crude fibre, 15.1 and 19.2, respectively. These figures are very different from those reported by Ghazalah and Ali (2008) and Polat *et al*. (2011) for rosemary leaves. Additionally, it has been observed that the essential oil content in rosemary leaves decreases over time, especially when they are stored in powdered form (Verma *et al*., 2011). Bimbirait *et al*. (2008) also found differences in the essential oil content and composition between the flowers and leaves of four yarrow morphotypes. However, it is presumably the case that studies carried out in optimum conditions involving highly digestible diets and clean conditions might not induce the improvement of growth-related parameters in broilers (Jang *et al*., 2007).

Carcass traits and organ weights

In the present work, no significant differences (P>0.05) between treatments were found in the final body weight or in most carcass traits (Table 3), in agreement with Sharifi *et al*. (2013) and Yakhkeshi *et al*. (2012). The breast weight relative to the carcass weight showed a quadratic response (P<0.05) to the rosemary supplementation level, the numerically highest value being observed in the R1.0 treatment. The carcass weight relative to the body weight and the drumstick weight relative to the carcass weight were lowered (P<0.05) by yarrow supplementation, compared with the control treatment. The relative weight of the liver increased (P<0.05), due to yarrow supplementation, compared with the control treatment, but the effect of yarrow was only clear at the highest supplementation level. The relative weight of the gastrointestinal tract increased (P<0.05), due to rosemary and yarrow supplementation, compared with the control treatment, and showed a positive linear response (P<0.001) to the rosemary supplementation level. Our results disagree with those of Cabuk *et al*. (2006) and Jang *et al*. (2007), who did not find any changes in the gastrointestinal tract weight in response to the inclusion of essential oil mixtures in broiler diets. The observed changes in the present work could be related to an effect of the herb supplements on the gastrointestinal tract. It could be expected that, if having any antimicrobial activity, plant feed additives would not have changed or would have even reduced the gut weight (Coates *et al*., 1955). However, the high crude fibre content in the rosemary and yarrow herb powders used in the present study could cause an expansion of the gastrointestinal tract (Jørgensen *et al*., 1996).

Ileal microbiota

The effects of rosemary and yarrow herb powders on ileal microbiota are shown in Table 4. No differences were observed in the *Lactobacilli* counts for the rosemary and yarrow supplementation levels at 21 and 42 days of age. However, the *Lactobacilli* counts showed a quadratic response (P<0.05) to the yarrow supplementation level at 21 days of age, and the *Lactobacilli* counts were linearly decreased (P<0.01) by the rosemary supplementation at 42 days of age. On the other hand, the rosemary supplementation increased (P<0.05) the *Lactobacilli* counts at

Table 3. Carcass traits and organ weights of 6-week old broilers fed diets containing either no plant feed additives, or 0.5%, 1.0% or 1.5% of rosemary or yarrow herb powders.

	Treatments										
	C	R			Y			SEM	P		
		R0.5	R1.0	R1.5	Y0.5	Y1.0	Y1.5		CxR	CxY	RxY
BW, g	2593	2767	2790	2747	2568	2647	2607	47.48	ns	ns	ns
Carcass weight, % BW	64.06	63.23	61.91	62.53	62.22	61.52	62.66	0.276	0.070	<0.05	ns
Breasts, % CW	37.82	35.61	38.00	36.48	36.06	34.79	37.03	0.306	ns	<0.05	ns
Drumsticks, % CW	27.69^{ab}	26.31^{b}	26.92^{ab}	27.00^{ab}	28.00^{ab}	28.59^{a}	27.66^{ab}	0.169	<0.05	NS	<0.001
Wings, % CW	8.50	8.94	8.36	8.49	8.69	8.47	9.08	0.096	ns	ns	ns
Organ weights, % BW											
Liver and bile	2.39^{b}	2.58^{ab}	2.45^{b}	2.55^{ab}	2.57^{ab}	2.41^{b}	2.90^{a}	0.039	ns	<0.05	ns
Gastrointestinal tract	7.06^{cd}	7.00^{d}	7.60^{bcd}	9.12^{a}	8.62^{ab}	8.16^{abc}	8.00^{bcd}	0.131	<0.05	<0.01	ns

BW, body weight. $^{a-d}$In a row, the least squares means with a different superscript differ significantly (P<0.05); ns, not significant.

Table 4. Ileal microbiota (log CFU/g digesta) at two ages in broilers fed diets containing either no plant feed additives, or 0.5%, 1.0% or 1.5% of rosemary or yarrow herb powders.

	Treatments										
	C	R			Y			SEM	P		
		R0.5	R1.0	R1.5	Y0.5	Y1.0	Y1.5		CxR	CxY	RxY
Lactobacilli											
21 d	7.63^{ab}	8.01^{ab}	8.29^{ab}	8.53^{a}	7.91^{ab}	8.34^{ab}	7.21^{b}	0.109	<0.05	ns	<0.05
42 d	7.07^{b}	8.09^{a}	7.91^{a}	7.41^{ab}	7.86^{ab}	7.75^{ab}	7.69^{ab}	0.080	<0.01	<0.01	NS
Escherichia coli											
21 d	7.53	6.86	7.32	7.93	7.49	7.46	7.50	0.102	ns	ns	ns
42 d	7.79^{a}	6.39^{b}	6.83^{ab}	7.52^{ab}	7.71^{a}	7.99^{a}	7.81^{a}	0.120	<0.01	ns	<0.001

$^{a-c}$In a row, the least squares means with a different superscript differ significantly (P<0.05).

21 days of age, compared with the control and yarrow treatments, and both herb supplements equally increased (P<0.01) the *Lactobacilli* counts at 42 days of age, compared with the control treatment. The *Escherichia coli* counts did not show any significant differences (P>0.05) between treatments at 21 days of age. Increasing the rosemary supplementation level linearly increased (P<0.001) the *Escherichia coli* counts at d 42. However, the rosemary supplementation decreased the *Escherichia coli* counts, compared with the control treatment, and to the yarrow supplementation (P<0.01 and P<0.001, respectively).

In contrast with our results, Al-Kassie *et al*. (2008) observed an upward trend and a downward trend in *Lactobacilli* counts and coliform counts, respectively, as the rosemary content increased in broiler feed from 0.5 to 1%. On the other hand, Cross *et al*. (2007) did not find any effects of rosemary or yarrow on lactic acid bacteria or coliform counts. Additionally, Sharifi *et al*. (2013) did not find any effect of yarrow on *Lactobacilli* or coliform counts. The finding that yarrow did not have any effect on *Escherichia coli* counts in the present work is in agreement with the results of Nascimento *et al*. (2000), who observed that yarrow extracts did not have any *in vitro* antimicrobial activity against several antibiotic susceptible and resistant microorganisms, while a rosemary extract showed a moderate effect. Moreover, Mathlouthi *et al*. (2012) found that rosemary essential oil was *in vitro* effective against *Escherichia coli*, although its antimicrobial activity was lower than that of oregano oil. It should be borne in mind that any increase in *Lactobacilli* counts and low coliform counts can be considered positive from the point of view of gut health (Jin *et al*., 1996).

Conclusions

Under the conditions of the present work, the inclusion of rosemary or yarrow herb powders in broiler feeds showed different but positive effects on some of the studied parameters. Yarrow supplementation mainly improved growth performance, while rosemary supplementation showed the best effects on ileal microbiota. Several of the observed responses were linearly or quadratically related to the rosemary and yarrow supplementation levels. More studies are needed to explain the mode of action of the active components of such plant extracts and to establish the appropriate level of supplementation.

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