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The effect of using rosemary (*Rosmarinus officinalis*) in broiler nutrition on production parameters, slaughter characteristics, and gut microbiological population

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Abstract: The aim of this study was to investigate the effects of the supplementation of different concentrations of rosemary in broiler diets from day 1 to 42 on growth performance, carcass quality, and cecal microbiological composition of chickens. The research was carried out with 1200 Ross 308 chickens, divided into 4 groups with 6 repetitions per group. The treatment groups were: control group (C) without rosemary, 0.2% rosemary group (0.2R), 0.4% rosemary group (0.4R), and 0.6% rosemary group (0.6R). Production parameters and the European Production Efficiency Factor (EPEF) were determined. At the end of the trial 12 chicks per group were selected and euthanised in order to determine the slaughtering properties and for the microbiological analysis of the cecum contents. The obtained results showed that the use of rosemary in diets leads to increase in average daily gain, feed conversion, and EPEF value. The share of abdominal fat in carcasses was lower ($P < 0.05$) in the 0.4R and 0.6R groups. Lactobacilli count was found higher in the 0.4R and 0.6R groups ($P < 0.001$) compared to the C group. These results demonstrate that addition of 0.4% rosemary powder to broiler feed could have positive effects on the gain, feed conversion, fat deposition, and cecal microbiological composition. Further increase in the amount of added rosemary had no additive effect in this research.

Key words: Broiler chickens, phytobiotics, rosemary, diet

1. Introduction

Consumer requirements for the highest quality livestock products should be achieved by the application of appropriate health-safe animal nutrition systems. In the European Union, the use of antibiotics in feed as promoters of growth has not been acceptable since 2006. Increased occurrence of antibiotic-resistant bacterial strains and strong consumer concerns have prompted other non-EU countries to follow EU directives regarding the use of antibiotics as growth promoters. This has increased the pressure on the poultry industry to find adequate alternatives. Probiotics (1), medicinal herbs (2), prebiotics, and organic acids can be used instead of antibiotics in animal nutrition.

In the last 20 years, phytobiotics have taken a significant role as promoters of growth and can be a way to reduce losses caused by antibiotics use reduction or ban. Rosemary and other phytobiotics are well known to have a very complex mixture of bioactive components that could improve production results and/or the health of animals. Phytogenic additives affect the growth of broiler chickens, feed consumption, and feed conversion (3,4). Windisch et al. (5) reported that phytogenic additives improve the microbiological status of the intestines (reduce the number of pathogens and increase the total number of beneficial bacteria), resulting in improved immunity, increased resistance to digestive tract diseases, and increased production performance. However, the effectiveness of phytobiotic additives, especially in natural form, can be very variable, affected by many factors: source of used herb, harvest time, used parts of the plant, physical properties, feed composition/interaction, etc. This can be part of the explanation for contradictory results of studies on the use of herbs in broiler nutrition and the constant need for further research on phytobiotics.

Rosemary (*Rosmarinus officinalis*), a perennial aromatic plant from the family Lamiaceae, contains resin, tannin, and small amounts of saponin. The antimicrobial and antioxidant activity of rosemary is well known, and among others, confirmed by authors (6,7). Phenolic compounds are responsible for antimicrobial action (8). The antioxidative activity of rosemary is due to phenolic

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terpenes, rosmarole, and rosmarinic acid (9), which leads to an improvement in the oxidative stability of poultry meat (10). The positive effects of the use of rosemary in the diet of broiler chickens (11) have been identified on production performance, immune status, and meat quality. Yesilbag et al. (12) cited the positive effects of using rosemary oil on quail laying capacity. Some reports suggested that rosemary could have significant influence on poultry nutrition and health, although feed concentrations and the form of the plant added to feed still need to be established. On the other hand, Loetscher et al. (13) reported that the addition of rosemary to chicken diets does not have a significant impact on production and slaughter results. However, the prevailing opinion is that rosemary has significant potential in the nutrition of poultry, but it is important to choose the proper source, form, concentration, and mode for practical application in broiler diets.

The aim of this study was to investigate the effects of the addition of different concentrations of locally grown rosemary powder in broiler chicken diets on production results, carcass quality, and the microflora of the digestive tract.

2. Materials and methods

2.1. Trial design and diet

The study was carried out with 1200 one-day-old broiler chickens (Ross 308 hybrid) and lasted 42 days. Chickens were divided into 4 treatments and housed in 24 cages, with 6 repetitions per group. Twenty-five male and 25 female broilers were placed in 2 × 2 m cages (0.08 m^2 per bird). Ambient temperature was set at 34 °C at placement and then decreased gradually to achieve 23 °C from day 24 onwards. In the first week, the light regime was 23 h of light (L) and 1 h of darkness (D) and after the first week it was below 18L:6D.

During the study, all broilers were fed ad libitum with complete corn/soy-based mesh-type diets (starter, grower, and finisher) formulated according to the recommendations used for hybrids and analyzed by proximal analysis in an accredited laboratory (Table 1). The diets for trial groups differed in the amount of rosemary powder additionally mixed in basal diets: control group (C) without rosemary, 0.2% rosemary group (0.2R), 0.4% rosemary group (0.4R), and 0.6% rosemary group (0.6R).

Rosemary plants were grown and processed (drying and grinding only) by a local producer (Banatski Karlovac, Serbia) with quality required for human use. Particle diameter of the used rosemary powder was 1 mm. Standard proximal chemical analysis showed that the used rosemary powder contained 89.1% dry matter, 9.4% crude protein, 4.6% crude fat, 17.5% crude fiber, and 12.8% ash.

2.2. Production parameters

Feed and birds at 10, 24, and 42 days of age were weighed to determine body weight, feed intake, and feed conversion rate. The European Production Efficiency Factor (EPEF) was established on the basis of the achieved average weight, vitality, food conversion, and fattening duration with the following formula: EPEF = (live weight, kg × livability, % / age of depletion, days × feed conversion ratio, kg feed / kg gain) × 100.

2.3. Slaughter characteristics

Twelve chickens for each treatment (1 male and 1 female chicken from each cage in each treatment) were selected (average body weight of the group was used as criterion), measured, and slaughtered after 4 h of starvation (48 chickens in total). Chickens were processed manually and then cooled to 4 °C for 24 h. After cooling, the carcass weight and the abdominal fat weight were determined, the carcasses were cut, and the main parts (breasts, drumsticks, thighs, and wings) were weighed. The weights of the liver, heart, and stomach were determined during the treatment of the carcasses.

2.4. Microbiological analysis of the cecal content

The cecal content was taken from slaughtered chickens, placed in sterile bottles, and taken to the laboratory as quickly as possible. Proper dilutions of cecal content were made in nutrient broth and within 1 h plated under aseptic conditions on selected media. Total aerobic bacterial count was determined on plate count agar (PCA) (Torlak) after incubation at 30 °C for 72 h in an aerobic environment. Total *Escherichia coli* count was determined on modified UTI agar (UTI agar, HiMedia) after incubation at 37 °C for 24 h in an aerobic environment. MRS agar (Becton Dickinson) was used to examine the presence and determine the total lactobacilli count at 37 °C for 72 h in an anaerobic environment.

2.5. Statistical analyses

The obtained results were analyzed using the statistical software package STATISTICA (StatSoft Inc., 2012). The methods of descriptive statistics, analysis of variance (one-way ANOVA), and post hoc LSD tests were used. Assessment of significance of the differences established was carried out at the probability level of 95% ($P < 0.05$).

Prior to statistical processing of obtained data for microbiological analyses of the cecum, their transformation was performed using the logarithmic function log10(x).

3. Results

The effects of adding rosemary herb in broiler chick nutrition on production performance are shown in Table 2. The use of rosemary during the starter and grower periods led to no differences in feed intake, average daily gain, and feed conversion ratio ($P > 0.05$). However, in the finishing period, rosemary addition in diets improved the feed

Table 1. Composition of the basal diets.

Ingredient, g/kg	Starter	Grower	Finisher
	0–10 days	11–24 days	25–42 days
Corn	499.9	547.4	593.9
Soybean meal	342	245	149
Full-fat soybeans (extruded)	100	150	200
Vegetable oil	20	20	20
Limestone	14	13.5	13
Monocalcium phosphate	12	11.5	11
Salt	2	2.5	3
Vitamin + mineral premix[1]	10	10	10
DL-methionine	0.1	0.1	0.1
Total	1000	1000	1000
Nutrients (analyzed) and energy level (calculated)			
ME, MJ/kg	12.6	13.0	13.4
Crude protein, %	23.1	21.2	18.9
Crude fat, %	6.23	7.13	8.41
Crude fibers, %	3.55	3.27	3.01
Lysine, %	1.32	1.16	1.00
Methionine, %	0.50	0.47	0.44
Calcium, %	0.90	0.85	0.80
Total P, %	0.70	0.68	0.66
Available P, %	0.44	0.42	0.40

[1]Contents per kilogram: vitamin A, 12000 IU; vitamin D3, 5000 IU; vitamin E, 50 mg; vitamin K3, 6 mg; thiamine, 3 mg; riboflavin, 9.4 mg; niacin, 5 mg; pantothenic acid, 10 mg; pyridoxine, 4 mg; folic acid, 1.5 mg; vitamin B12, 0.02 mg; biotin, 0.02 mg; choline, 400 mg; Mn, 100 mg; Fe, 30 mg; Zn, 100 mg; Cu, 8 mg; I, 0.5 mg; Se, 0.2 mg.

conversion ratio ($P < 0.05$). The 0.4R and 0.6R groups ($P < 0.05$) had better feed conversion ratios (FCRs) compared to the C and 0.2R groups. Similar findings were obtained for the whole experimental period. The increase of the addition of rosemary in the mixtures at 0.4% or 0.6% levels significantly increased the average daily gain and improved FCRs ($P < 0.01$) when the entire experimental period is observed. Finally, as a result of better feed conversion, higher daily gain, and insignificantly lower mortality of chickens in groups 0.4R and 0.6R, significantly higher ($P < 0.05$) values of EPEF compared to the C and 0.2R groups were determined. On the other hand, chickens fed with 0.4% and 0.6% rosemary in diets had similar values of all determined production parameters during the trial, indicating that birds did not respond to further increase of rosemary over 0.4%.

The results of slaughter performance of broiler chicks fed with the addition of different rosemary concentrations are shown in Table 3. Body weights before slaughter, dressing yields, and shares of drumsticks, thighs, wings, heart, liver, and stomach did not differ significantly under the influence of the examined factor. Significant differences ($P < 0.05$) were established for the share of breast in chicken carcasses of the 0.6R group in comparison with the 0.2R group, but not with the C group. The 0.4R and 0.6R groups ($P < 0.05$) had less abdominal fat compared to the C group. Nominally, the final product of broiler fattening, the chicken carcass, had 0.35% less fat and about 1% more breast meat in groups fed 0.4% or more added rosemary in feed.

The effects of consumption of rosemary powder on the results of the microbiological analysis of the cecal content of chickens are shown in Table 4. The total number of aerobic bacteria, as well as the *Escherichia coli* count, did not differ significantly between groups. Significant differences ($P < 0.001$) were determined for the lactobacilli count, where a

Table 2. Production performance.

	Treatments				SEM	P
	C	0.2R	0.4R	0.6R		
Starter period (1–10 days)						
FI, g/day	21.25	20.70	21.40	21.45	0.177	0.456
ADG, g/day	14.52	14.46	14.92	15.20	0.200	0.563
FCR, g/g	1.46	1.43	1.44	1.41	0.014	0.659
Grower period (11–24 days)						
FI, g/day	72.67	73.96	72.32	74.63	0.515	0.372
ADG, g/day	45.78	46.51	45.98	47.18	0.482	0.777
FCR, g/g	1.59	1.59	1.57	1.58	0.013	0.972
Finisher period (25–42 days)						
FI, g/day	140.00	141.06	140.60	137.68	0.871	0.571
ADG, g/day	72.79	73.92	76.50	75.47	0.610	0.132
FCR, g/g	1.92[a]	1.91[a]	1.84[b]	1.82[b]	0.016	<0.05
Whole period (1–42 days)						
FI, g/day	89.01	90.09	89.17	89.02	0.364	0.723
ADG, g/day	49.91[b]	50.63[b]	51.66[a]	51.69[a]	0.237	<0.01
FCR, g/g	1.79[a]	1.78[a]	1.73[b]	1.72[b]	0.011	<0.05
Mortality, %	1.00	1.00	0.50	0.50	0.359	0.938
EPEF	281.89[b]	287.45[b]	302.82[a]	304.12[a]	3.343	<0.05

C, Control; FI, feed intake; ADG, average daily gain; FCR, feed conversion rate; [a-b] In a row, the least squares means with different superscripts differ significantly ($P < 0.05$).

Table 3. Slaughter traits of broiler chickens.

	Treatments				SEM	P
	C	0.2R	0.4R	0.6R		
BW, g	2116.7	2125.0	2220.8	2227.5	32.370	0.475
Carcass weight, % BW	67.10	68.22	68.16	68.44	0.221	0.135
Breasts, % CW	29.67[ab]	29.25[b]	30.46[ab]	30.95[a]	0.241	<0.05
Thigh, % CW	15.30	15.46	15.08	15.25	0.141	0.833
Drumsticks, % CW	17.21	17.37	17.44	17.15	0.107	0.756
Wings, % CW	11.65	11.47	11.50	11.80	0.094	0.605
Abdominal fat, % CW	1.01[a]	0.83[ab]	0.74[b]	0.76[b]	0.038	<0.05
Organ weights, % BW						
Heart	0.50	0.50	0.52	0.56	0.013	0.335
Liver	1.79	1.71	1.72	1.77	0.038	0.884
Gizzard	1.89	1.87	1.98	1.98	0.028	0.355

BW, Body weight; CW, carcass weight; [a-b] In a row, the least squares means with different superscripts differ significantly ($P < 0.05$).

Table 4. Microbiological analysis of cecal content, log10 CFU/g.

	Treatments				SEM	P
	C	0.2R	0.4R	0.6R		
Total number of aerobic bacteria	8.67	8.69	8.44	8.40	0.077	0.406
Escherichia coli	6.80	6.54	6.59	6.51	0.069	0.472
Lactobacilli	7.38[b]	7.54[ab]	7.74[a]	7.87[a]	0.048	<0.001

[a-b] In a row, the least squares means with different superscripts differ significantly ($P < 0.05$).

lower number was found in the C group than the 0.4R and 0.6R groups. With the increase in rosemary concentration, the number of lactobacilli increased gradually.

4. Discussion

Supplementation of broiler diets with herbs must be first considered concerning possible dilution, antinutritive, or other negative effects on the extremely fast production. Rosemary has a strong flavor and high contents of crude fiber, tannin, and other potentially interfering substances, which can limit the amount of rosemary in feed (13). The used rosemary concentrations in feed in our study were relatively low and showed no negative effects on the birds.

In our study, 0.4 and 0.6% supplemented rosemary powder in broiler feed improved average daily gain, FCRs, and EPEF values. The improvement in chicken production performance as a consequence of the use of rosemary in rations was also confirmed by the results of Al-Kassie et al. (14) with the addition of 0.5% and 1% rosemary. Norouzi et al. (15) confirmed the positive effects of using different concentrations of rosemary powder on the feed conversion of chickens to 42 days of age, but did not determine significant differences in feed consumption or in gain. Yesilbag et al. (16) reported that the use of rosemary caused significant differences in average daily gain, feed conversion, and slaughter yield of chickens, with better results achieved by using essential oil of rosemary in relation to the whole plant. Ghazalah and Ali (11), with the use of rosemary powder at a concentration of 0.5% in food mixtures, achieved a significantly higher gain and better conversion of feed compared to groups with higher share of rosemary. As the authors explained, increasing rosemary concentration also increases the level of crude fiber contents in the mixture, which interferes with the utilization of nutrients. Similar results were also obtained by Rostami et al. (17). However, the exact growth-promoting mechanisms when using rosemary in broiler nutrition are still insufficiently understood (except, to some extent, through its antimicrobial and antioxidant activity) and need to be further investigated. The positive effect of rosemary on the production results in this research was probably achieved primarily through the antimicrobial and antioxidant activity of the rosemary in the digestive tract of the birds, with possible antiinflammatory and hepatoprotective effects. Such effects have been achieved mostly in studies with essential oils of rosemary, but it can be assumed that similar effects can be created with long-lasting use of high-quality herbs.

This study found that supplementation of rosemary in broiler diets does not affect carcass yield, but could, to some extent, improve carcass quality. Similar slaughter results with the use of rosemary powder in chicken diets were obtained by Ghazalah and Ali (11), who argued that slaughter yields and liver and heart shares did not differ significantly between groups, although a lower proportion of abdominal fat was determined in chickens fed mixtures with the addition of 0.5% rosemary compared to the control. Norouzi et al. (15) also found no significant differences between groups in the slaughter yields and shares of breast, thighs, and wings. Zhang et al. (18) found that in broilers fed mixtures with the addition of various essential oils, there were no significant differences in the share of the breast and drumstick weights in the carcass weight compared to the control group. Hernández et al. (19) did not determine the effect of using different plant extracts on the weights of the stomach, liver, and pancreas. Milošević et al. (20) stated a significantly higher proportion of abdominal fat in broilers fed mixtures with the addition of phytobiotics.

The results obtained in this study regarding improved microbiological composition of the cecum can be compared with the study of Norouzi et al. (15), who, with the use of 0.5% rosemary in nutrition of broilers, obtained significantly better results in terms of the number of lactobacilli and *Escherichia coli* (at the age of 42 days) not only compared to the control (0%) but also groups with higher concentrations of the tested additive (1% and 1.5%). Murugesan et al. (21) found greater lactobacilli counts in the ceca of broilers that consumed mixtures with the addition of phytogenic additives compared to mixtures with antibiotic growth promoters and a control group. Jin et al. (22) stated that the presence of lactobacilli inhibits the growth of *Escherichia coli*. In addition, Rahimi et al. (23) found an increase in the number of lactobacilli and a decrease in *Escherichia coli* in the intestines of broilers that consumed diets with a mixture of essential oils. Contrarily, Cross et al. (24) stated that when using a mixture of essential oils, there was no change in the number of lactobacilli in the cecum. Al-Kassie et al. (14) found a significant reduction in the total number of bacteria in the small and large intestine (colon) with the use of rosemary at a concentration of 1%. However, the findings of some reports, including this study, suggest that rosemary in feed could improve the microbiological status of the intestines (increase the total number of beneficial bacteria and/or reduce the number of pathogens), which could lead to improved immunity, increased resistance to gut diseases, and better digestibility of the nutrients.

On the basis of the results obtained in this study, it can be concluded that the addition of 0.4% dried and finely ground rosemary to the complete corn/soy-based mesh diets for broilers from the first day of fattening has positive effects on the daily gain and feed conversion at the end of the production, as well as carcass quality and microbiological composition of the cecum. Further increase in the amount of added rosemary had no additive effect in this research.

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