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Effect of *Arbutus pavarii*, *Salvia officinalis* and *Zizyphus Vulgaris* on growth performance and intestinal bacterial count of broiler chickens

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Abstract A study was conducted to determine the effect of three native plants from El-Jabal al ELAkhdar, (Libya) on performance and cecal coliform count of broiler chickens. A total of 1260 one-day-old male Cobb chickens were used in the experiment. The birds were assigned to 7 treatment groups (6 replicates per treatment). The dietary treatments included basal diet with no additive (control), and 6 other dietary treatments (*Arbutus pavarii*, *Salvia officinalis* and *Zizyphus Vulgaris*) each of which was added at the rate of 0.5 g and 1 g/kg of basal diet. Results explicitly revealed that all dietary treatments had a significant effect on body performance of broiler chickens compared to the control with the exception of the dietary treatment of *S. officinalis* at dosage of 0.5 g/kg that has expressed noticeable reduction in body weight. Coliform counts in the cecum of birds receiving 1% *A. pavarii* and 1% *Z. Vulgaris* were significantly lower ($P \leqslant 0.05$) than those of control group from early weeks of treatments, whereas all plant shows a significant lowering ($P \leqslant 0.05$) of cecal coliform count during the rest of experiment compared to control group. These results emphasize the potential biotic role of such plants together with the immune modulating effects on treated birds. However, further pharmacological and clinical work should be adopted in the future to present an obvious understandable theory behind the potential beneficial as well as side effects of such natural plants.

1. Introduction

Antibiotics are widely used as feed additives in poultry industry for purposes of improving health and performance [1]. However, the concerns about developing antibiotic resistant

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Table 1 Diet analysis for all stages of the experiments.

Ingredients %	Starter	Grower (1)	Grower (2)	Finisher
Yellow corn	62.10	65.85	68.89	68.89
Soybean meal	33.00	28.04	25.10	25.10
Soybean oil	00.50	01.60	01.90	01.90
Salicylate	00.15	00.15	00.15	00.15
Methionine	00.23	00.14	00.15	00.15
Lysine	00.14	00.13	00.13	00.13
Choline chloride	00.08	00.10	00.12	00.12
Vitamin premix	00.50	–	–	–
Dicalcium phosphate	01.58	01.55	01.37	01.37
Salt (NaCl)	00.20	00.25	00.26	00.26
Monocalcium Phosphate	01.52	01.66	01.40	01.40
Mineral premix	–	00.50	00.50	00.50

bacteria in human have led the European Union and the United States to pass legislation to ban the usage of antibiotics as growth promoter. Consequently, the research now has been focused on natural products such as plants or their extracts as possible alternative to antibiotics [2]. Plants and their extracts are known to possess many bioactive components such as tannin, alkaloids, and essential oils which have both antimicrobial and antioxidant activities [3–5]. These bioactive components exert their beneficial effects by manipulating the intestinal microflora and improving digestibility. Furthermore, many studies have indicated that plants and their bioactive components can decrease intestinal pH thus favoring the growth of beneficial bacteria such as lactobacilli and bifidobacteria and reducing the number of the coliforms and *Clostridium perfringens* in the ileum and cecum [6,7]. This can stabilize the gut microflora and provide a protection against pathogenic bacteria [8–10].

The *Arbutus pavarii* is an endemic plant in eastern mountain of Libya and it is being used as food, honey production and treatment of some particularly kidney diseases. It is known to contain important antioxidant components such as flavonoids, tannins, glycosides, simple phenolics [11,12]. *Salvia officinalis* is a plant endemic in Mediterranean countries with great medical importance. It has strong antioxidant activity due to the presence of rosmarinic and carnosic acids in high amount and it also exhibits activity against many bacterial species [13,14]. The root extract of *Zizyphus vulgaris* was found to exhibit activity against *Staphylococcus aureus* and *Escherichia coli* [15].

Abouzeed et al. [16] have recently reported an *in-vitro* antibacterial activity of *A. pavarii S. officinalis* against *Staphylococcus aureus* MRSA strain. The reported results showed significant efficacy of these plants against *S. aureus* MRSA. In addition the tested plants have showed antioxidant activity. In Libya, there are no available data on the commercial or experimental use of plants or their extracts as feed additives for animals. Only the effect of *S. officinalis* extract on growth performance of broilers was studied and the results were found to be irrelevant [17]. We have speculated that the whole plant might have potential effect on growth performance than did the extracts and could be a possible alternative to antibiotic feed additives; however, their efficacy needs to be evaluated. Consequently, the objective of the present study was to determine the effects of different levels of *A. pavarii*, *S. officinalis* and *Z. Vulgaris* on growth performance and intestinal bacterial count of broiler chickens.

2. Materials and methods

2.1. Chicken, housing and diet

A total of 1260 one day old male Cobb chickens (Cobb Germany) were used in the experiment The birds were wing tagged, weighed and placed in floor pens with a wood-shaving floor (30 birds per pen; size: 2.3×1.2 m). The birds were assigned to 7 treatment groups (6 replicates per treatment). The dietary treatments included basal diet with no additive (control), and 6 other dietary treatments (*A. pavarii*, *S. officinalis* and *Z. Vulgaris*) each of which was added at the rate of 0.5 g and 1 g/kg of basal diet. The plants were collected from El-Jabal al ELAkhdar, Libya. The whole aerial parts of the plants were air-dried and ground to coarse powder. The diets were manufactured at a commercial company. The ingredient and composition of basal diet are presented in Table 1. The feeding program consisted of a pre-starter diet fed from day one to day 14 and a finisher diet fed from day 15 to day 42. Water and feed were available ad libitum and the temperature was gradually decreased from 37 °C to 25 °C till the end of the trial (42 days). All birds were vaccinated according to the vaccination program implemented by the Department of Animal Welfare, Libya. The experiment was conducted at the farm of Faculty of Agriculture, University of Tripoli. Live body weight, feed intake and feed conversion ratio were measured on weekly basis. The body weight and feed intake were determined weekly to each replicate.

2.2. Performance parameters of birds

Live body weight (LBW) was determined on weekly basis and feed conversion ratio (FCR) was calculated.

2.3. Bacteriological examination

The intestinal bacterial populations were determined at 7, 14, 21, 28, 35 and 42 day old. Approximately 1 g of the cecal contents was mixed with 9 ml of sterile peptone water and homogenized for 3 min. From the initial 10^{-1} dilution, 10-fold serial

Table 2 Effect of *A. Pavarii, S. Officinalis* and *Z. Vulgaris* on body weight gain in broiler chickens.

Treatments (g/1 kg diet)							
Measurement per week	Control	*A. Pavarii*		*S. Officinalis*		*Z. Vulgaris*	
		0.5	1	0.5	1	0.5	1
1st w	8.32 ± 206.25[a]	5.34 ± 197.83[b]	6.62 ± 197.50[b]	9.36 ± 194.41[b]	6.60 ± 195.58[b]	6.06 ± 196.83[b]	5.64 ± 199.08[b]
2nd w	4.93 ± 450.83[a]	5.49 ± 438.66[a,b]	3.99 ± 431.58[b,c]	7.52 ± 358.50[b]	6.54 ± 441.33[a,b]	3.12 ± 423.00[c]	2.57 ± 432.75[b,c]
3rd w	31.10 ± 843.83[a]	23.30 ± 850.83[a]	7.93 ± 855.75[a]	14.39 ± 668.00[b]	25.07 ± 875.58[a]	18.08 ± 855.58[a]	25.64 ± 860.50[a]
4th w	21.56 ± 1445.83[a]	26.80 ± 1316.00[b]	44.66 ± 1357.33[b]	27.28 ± 1066.00[c]	19.07 ± 1378.00[a,b]	25.61 ± 1380.50[a,b]	24.21 ± 1315.60[b]
5th w	65.61 ± 2046.50[a]	39.15 ± 1987.83[a]	56.61 ± 2014.92[a]	42.23 ± 1570.83[b]	27.73 ± 2042.92[a]	16.98 ± 2019.75[a]	39.09 ± 1948.62[a]
6th w	23.57 ± 2466.20[a,b]	78.57 ± 2326.08[b]	95.32 ± 2422.92[a,b]	58.51 ± 2059.08[b]	58.75 ± 2584.38[a]	57.71 ± 2589.35[a]	63.98 ± 2455.86[a,b]

[a,b,c] = means on the same row have the same letter are not significantly different ($P \leqslant 0.05$).

Table 3 Effect of *A. Pavarii, S. Officinalis* and *Z. Vulgaris* on daily feed consumption ratio in broiler chickens.

Treatments (g/1 kg diet)							
Measurement per week	Control	*A. Pavarii*		*S. Officinalis*		*Z. Vulgaris*	
		0.5	1	0.5	1	0.5	1
1st w	21.33 ± 1.20[a,b]	23.84 ± 0.29[b,c]	25.89 ± 1.19[a,b]	27.01 ± 1.14[a]	24.10 ± 0.62[b]	28.09 ± 0.95[a]	27.20 ± 1.02[a]
2nd w	36.72 ± 2.92[a]	41.86 ± 5.10[a]	37.23 ± 2.96[a]	34.22 ± 5.10[a]	37.90 ± 3.62[a]	35.72 ± 4.11[a]	34.72 ± 5.30[a]
3rd w	84.85 ± 1.85[a]	85.46 ± 5.34[a]	80.27 ± 1.75[a]	60.98 ± 5.67[b]	86.61 ± 5.95[a]	87.22 ± 5.74[a]	80.05 ± 1.68[a]
4th w	117.91 ± 9.10[a]	100.23 ± 8.30[a]	116.34 ± 8.39[a]	71.38 ± 9.50[b]	116.37 ± 9.71[a]	101.90 ± 11.39[a]	113.20 ± 3.10[a]
5th w	149.01 ± 9.08[a]	140.40 ± 3.35[a,b]	146.78 ± 1.35[a]	117.28 ± 19.66[b]	145.61 ± 1.62[a]	144.71 ± 2.19[a]	139.65 ± 7.78[a,b]
6th w	144.83 ± 13.20[a]	105.40 ± 9.82[c]	119.69 ± 15.07[a,b,c]	97.15 ± 21.13[c]	129.61 ± 9.14[a,b,c]	139.50 ± 14.90[a]	124.22 ± 11.37[a,b,c]

[a,b,c] = means on the same row have the same letter are not significantly different ($P \leqslant 0.05$).

Table 4 Effect of *A. Pavarii, S. officinalis* and *Z. Vulgaris* on feed conversion ratio of broiler chickens.

Treatments (g/1 kg diet)							
Measurement per week	Control	*A. Pavarii*		*S. Officinalis*		*Z. Vulgaris*	
		0.5	1	0.5	1	0.5	1
1st w	0.79 ± 0.06[b]	0.92 ± 0.04[c]	1.01 ± 0.04[a,b,c]	1.09 ± 0.04[a]	0.96 ± 0.06[b,c]	1.10 ± 0.05[c]	1.05 ± 0.06[c]
2nd w	1.05 ± 0.09[b]	1.23 ± 0.16[a,b]	1.12 ± 0.11[b]	1.25 ± 0.15[a,b]	1.09 ± 0.13[b]	1.11 ± 0.12[b]	1.01 ± 0.14[b]
3rd w	1.54 ± 0.09[a]	1.49 ± 0.16[a]	1.32 ± 0.03[a]	1.49 ± 0.12[a]	1.47 ± 0.07[a]	1.41 ± 0.22[a]	1.33 ± 0.07[a]
4th w	1.39 ± 0.14[a]	1.52 ± 0.10[a]	1.62 ± 0.12[a]	1.30 ± 0.12[a]	1.62 ± 0.12[a]	1.41 ± 0.22[a]	1.78 ± 0.12[a]
5th w	1.80 ± 0.18[b]	1.47 ± 0.04[b]	1.58 ± 0.07[b]	1.79 ± 0.19[b]	1.54 ± 0.04[b]	1.59 ± 0.05[b]	1.55 ± 0.07[b]
6th w	2.90 ± 0.29[a]	2.20 ± 0.23[a,b]	2.92 ± 0.21[a]	1.88 ± 0.32[b]	2.01 ± 0.25[b]	2.04 ± 0.34[b]	2.06 ± 0.27[b]

[a,b,c] = means on the same row have the same letter are not significantly different ($P \leqslant 0.05$).

dilutions were subsequently made in 0.1% peptone for aerobic bacteria. The samples form cecum were diluted to 10^{-5}, 10^{-7}, and10^{-9}. For each dilution 0.1 ml was inoculated in three plate of brain heart infusion for total aerobic bacterial count, MacConkey agar for coliforms bacteria. The total numbers of bacterial colonies were counted at 24 h. The laboratory procedures used to determine the total bacterial count and Coliform counts were done according to Salanitro et al. [18]; Iannotti et al.[19]; Dawson et al. [20].

For the measurements of the above mentioned parameters, 1 bird from each replicates was randomly selected. Total number of 6 birds cecal samples were pooled to form each collection per treatment.

2.4. Statistical analysis

All data were gathered and analyzed using Duncan test. This experiment was designed by Randomized Complete Block Design (RCBD). Data were analyzed using Statistical Analysis System (SAS, 2002) utilizing the analysis of variance. Duncan's multiple range test was used to compare between means.

3. Results

The effect of *Z. Vulgaris*, *S. officinalis* and *A. pavarii* supplementation on live body weight of broiler chickens is presented in Table 2. The results indicated that all dietary treatments have showed significant ($P \leqslant 0.05$) decrease on live body weight (LBW) in the first week compared to the control. Furthermore, continued reduction on the LBW was observed in the second week in 0.5% *S. officinalis*, 0.5% *Z. Vulgaris*, 1% *Z. Vulgaris* and 1% *A. pavarii*.

It is noteworthy to mention that the addition of 0.5% *S. officinalis* to diets during the third, fourth and the fifth week of age had significantly decreased the body weight ($P \leqslant 0.05$) compared to the control and the other treatments (Table 2). LBW of all the treatment groups were similar to the control as the trial approaching the sixth week (42 days) except of the 0.5% *S. officinalis* which had the lowest LBW, 2059.03 g compared to the control which was 2460.20 g.

There was a significant decrease ($P \leqslant 0.05$) in feed consumption ratio of the 0.5% *S. officinalis* compared to the other treatments and the control at 3, 4, 5 and 6 week (Table 3). At the end of the trial (42 days) there was also significant decrease ($P \leqslant 0.05$) in the feed consumption ratio for the 0.5% *A. pavarii* (105.40 g ± 9.82) compared to the control which was (144.83 g ± 13.20). Data indicated that feed consumption was declined during the last two weeks of trial among all treated groups when compared to the control group.

Table 4 shows the daily feed conversion ratio which indicated a significant increase ($P \leqslant 0.05$) in food conversion ratio at first week of administration of three types of plants with both concentrations. However, there has been an improvement in the feed conversion ratio during the 2nd up to the 5th week of trial in almost all treatment groups as compared to the control. At the 6th week there was a significant decline ($P \leqslant 0.05$) in feed conversion ratio in groups treated with *Salvia officinalis*, *Zizyphus Vulgaris* in both concentrations.

Table 5 shows the results of ceacal coliform count which indicated that the treatment with 1% of (*A. pavarii* and *Z. Vulgaris*) has significantly decreased ($P \leqslant 0.05$) the coliform count in the cecum of chicks from the first week of administration whereas, the three types of plants with both concentration showed a significant reduction in coliform counts compared with control groups from the 2nd till the end of the experiment.

Table 5 Effect of plant supplementation on Coliform count (cfu/g).

Treatments (g/1 kg diet)							
Treatment Period	Control	*A. Pavarii*		*S. Officinalis*		*Z. Vulgaris*	
		0.5	1	0.5	1	0.5	1
1st w	7.63 ± 0.55[a]	7.91 ± 0.01[a]	6.64 ± 0.11[b]	7.51 ± 0.10[a]	7.81 ± 0.03[a]	7.04 ± 0.03[a]	6.44 ± 0.03[b]
2nd w	7.52 ± 0.15[a]	6.02 ± 0.65[b]	6.44 ± 0.03[b]	6.46 ± 0.06[b]	6.38 ± 0.06[b]	6.44 ± 0.03[b]	6.27 ± 0.11[b]
3rd w	7.69 ± 0.11[a]	6.03 ± 0.08[b]	6.61 ± 0.07[b]	5.64 ± 0.12[c]	5.37 ± 0.06[c]	5.60 ± 0.05[c]	5.77 ± 0.07[c]
4th w	7.57 ± 0.14[a]	6.22 ± 0.02[b]	6.77 ± 0.07[b]	6.44 ± 0.03[b]	6.10 ± 0.17[b]	6.54 ± 0.27[b]	6.10 ± 0.06[b]
5th w	7.57 ± 0.06[a]	6.44 ± 0.03[b]	6.39 ± 0.08[b]	6.55 ± 0.04[b]	6.58 ± 0.03[b]	6.57 ± 0.02[b]	5.90 ± 0.05[c]
6th w	7.73 ± 0.03[a]	6.06 ± 0.01[b]	5.62 ± 0.02[c]	6.69 ± 0.04[b]	6.39 ± 0.02[b]	6.21 ± 0.04[b]	5.59 ± 0.02[c]

[a,b,c] = means on the same row have the same letter are not significantly different ($P \leqslant 0.05$).

4. Discussion

Data obtained in this study showed that all dietary treatments had significant effects on body performance of broiler chickens compared to the control with the exception of the dietary treatment of 0.5 g/kg *S. officinalis* which have showed noticeable reduction in body weight. A significant reduction in feed intake and improved feed conversion ratios were observed among all treatment groups compared to control. The significant reduction in body weight in the group fed on diet supplemented with 5% *S. officinalis* is odd and cannot be explained because there was no such effect in treatment group supplemented with 1% *S. officinalis*. Yurtseven et al. [17] have reported that the addition of sage extract to the poultry diet had no effect on performance yet it activates the liver antioxidant enzymes. It was also reported that the addition of 6% carnosic acid had significantly reduced the lipid peroxide in the liver [21]. This may explain the observation that certain amount of *S. officinalis* extracts such as carnosic acid may affect the metabolic activity and thus can influence the weight. In literature, there were no available data about the role of *S. officinalis* on weight loss. The extract of *S. officinalis* was found to element most of intestinal bacteria. In agreement with such observation, our results have indicated that the addition of *A. pavarii*, *S. officinalis* and *Z. Vulgaris* at level of 0.5% or 1% had significantly reduced the Coliform counts in cecum of chickens. Our results also indicated that the use of 1% *A. pavarii* and 1% *Z. Vulgaris* can decrease the coliform counts from first weeks of treatments significantly.

In conclusion, the enhancement of the growth performance is largely attributed to the bioactive compounds that present in those plants. The plants used in this study are known to contain poly phenolic compounds which known to have both antimicrobial and antioxidant activity. These bioactive compounds enhance the growth by increasing the digestibility of the nutrients and also by stabilizing the ecosystem of intestinal microflora. Our data showed that the feed intake was significantly decreased during the last weeks of trial among all treatment groups compared to the control. Further studies to investigate the relationship, effective dosages of *S. officinalis* and the loss of body weight at mammalian model are in real need to be adopted. These future studies would give better and accurate information about the correct dosage/beneficial usage of these plants. The other two plants could be used as supplements to the broiler to enhance the performance and also to improve the health status.

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