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THE EFFECT OF DIETARY SUPPLEMENTED DRIED FENNEL AND ROSEMARY ON THE PERFORMANCE AND CAECAL MICROFLORA OF GROWING RABBITS

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ABSTRACT: The objective of this study is to evaluate the possible effect of fennel and rosemary dietary supplements on the performance of rabbits. Therefore fifty-six weaned rabbits (40 days old) from white New Zealand breed were divided into two groups and submitted to the following dietary treatments: A) control diet and B) FR diet consisted of 2.5% *Foeniculum vulgare* seeds (fennel) and *Rosmarinus officinalis* leaves (Rosmary) as powder mixed by control diet for thirty days. The growth rate, feed conversion ratio, carcass yield, and mortality were not influenced by dietary fennel and rosemary supplementation. The antimicrobial effect of fennel and rosemary (2.5%) was not observed against *E. coli* in the caecum of the rabbit treated. The essential oils of *Foeniculum vulgare* and *Rosmarinus officinalis* are predominantly composed by Trans Anethole and 1-8 cineole successively. Low antibacterial activity was observed with two essential oils against the strain tested in this study. The addition of the 2.5% combination of fennel and rosemary in rabbit feed did not influence the zootechnical parameters of the rabbits

Keywords: Caecal microflora, Fennel, Growth parameters, Rabbit, Rosemary.

INTRODUCTION

In recent years, some interesting medicinal and aromatic plants in animal nutrition have emerged. The excessive use of antibiotics in feed animals results in bacterial resistance and antibiotic residues in animal products (Oliveira et al., 2020). Phytobiotics represent a part of plants or their bioactive compounds that can be used to improve animal productivity and the quality of food products derived from these animals without danger (Windisch et al., 2008; Alagawany et al., 2021; Ushakova et al., 2021). Their modes of action are still limited. However, antimicrobial activity, antioxidant activity, and the growth performance of phytobiotics were indicated by a lot of studies (Ambar et al., 2018; Łapiński et al., 2018; Aljumaah et al., 2021). Al-Snafi (2018) has reported that *Foeniculum vulgar*e treat abdominal cramps and spastic gastrointestinal disturbances. This aromatic plant has antibacterial, antiviral and antifungal activity (Badgujar et al., 2014). Yan et al. (2010) have showed that the average daily gain (ADG) and feed conversion ratio (FCR) significantly improved during the growing period of pigs using diet supplemented with essential oils rosemary.

Herein, in present study the efficacy of two medicinal plants as feed additives in the diet of rabbit was evaluated.

MATERIAL AND METHODS

Animals and experimental procedure

A total of 56 weaned rabbits white New Zealand (40 days old; male and female; 900 ± 100 g initial body weight), were divided into two groups and submitted to the following dietary treatments (Table 1) for twenty-one days. The first group was control diet, the second group was FR (2.5% *Foeniculum vulgare* and *Rosmarinus officinalis* powder mixed with the control diet). The two groups take only the control food for the remaining ten days.

The rabbits were kept in standard cages with 2 animals per cage (2010/63/EU Official Journal of the EU 2010) in a building with temperatures between 15 and 20°C, and humidity levels between 60% and 70%. The duration of daily illumination was 16 h. The rabbits had access to feed and water ad libitum. The bodyweight of rabbit's feeds consumption and mortality rates were measured every week during the experiment.

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Table 1 - Ingredients and chemical composition and nutritive value of basal diets

Ingredients	Control diet (%)	Fennel and Rosemary diet (%)	Chemical composition (g/100 g)	Control diet	Fennel and Rosemary diet
Bran	29	28.275	Dry matter	89.92	89.87
Corn	10	9.75	Ash	8.03	8.06
Soybean meal	4	3.9	Crude protein	16.77	16.7
Sunflower meal	5	4.875	Ether extract	2.66	2.82
Alfalfa	40.5	39.487	Crude fiber	14.5	14.7
Barley	9	8.775	Neutral detergent fibre	31.7	32
Salt	0.5	0.48	Acid detergent fibre	18.1	18.5
Premix *	1	0.975	detergent lignin	4.77	5.01
DL Methionine	0.05	0.048	Digestible energy (Kcal/Kg)	2270	2274
L-Lysine	0.25	0.24			
Dicalcium phosphate	0.61	0.59			
Calcium carbonate	0.09	0.08			
Fennel	0	1.25			
Rosemary	0	1.25			

*One kilogram of Premix provides: 1000000 IU vit.A, 300000 IU vit. D, 2 g vit. E, 0.4 g vit. K, 0.075 g vit. B1, 247 0.4 g vit. B2, 1.218 g vit. B3, 0.099 g vit. B5, 0.083 g vit. B6 , 0.190 g vit. B9, 0.030 g vit. B12, 0.005 g Biotin, 248 0,2 g Cuivre, 4 g Fer, 5 g Zinc, 0.012 g Iode, 0.012 g Selenium, 0.020g Cobalt, 6 g Manganese, 57 g Choline 249 chloride and QSP calcium. Premix contained 50 ppm of Salinomycin.

Chemical analysis

Chemical analysis of diets was calculated with Spanish foundation for the development of animal nutrition (Fundación Española para el Desarrollo de la Nutrición Animal, FEDNA) table of composition and nutritive value of ailments (Blas et al., 2010). The essential oils (EO) of each plant were extracted via steam distillation for 3 hours using a Clevenger-type apparatus. Chemical analysis of essential oils was done by gas chromatography coupled with mass spectrometry.

Bacteriological analysis

Bacterial counts

Three animals from each group were slaughtered on 40, 50, and 60 days. Bacteria from caecal samples were isolated by the standard microbiological method using the appropriate dilutions in the Ringer solution. Dilutions were plated onto the Mac Conkey agar for *E. coli*, incubated at 37°C for 24 hours. The bacterial counts were expressed in colony-forming units per gram (log 10 CFU · g–1).

Isolation of E. coli bacteria

Specimens collected from the carcasses of rabbits (not submitted to previous dietary treatment) showed prior signs of diarrhea and bloating. Miniature biochemical tests can be conveniently and simultaneously performed on a colony for *E. coli* identification using an API 20 e gallery.

Antibacterial activity

A preliminary assay was performed with the agar diffusion method to compare the antibacterial effects of the essential oils against the performance of the antibiotic Oxytetracycline. The diameters of the resulting inhibition zones were measured in centimeters, including the diameter of the well. The minimum inhibitory concentration (MIC) was defined as the low essential oil concentration capable of producing a total inhibition of growth after an incubation period of 24 to 48 hours (Remmal et al., 1993). The minimum bactericidal concentration (MBC) was defined as the minimum bactericidal concentration of the oil capable of killing the inoculum. The MIC1 and MBC2 values were determined by micro broth dilution assay using resazurin as 80 an indicator (Mann and Markham, 1998) of bacterial growth.

Statistical analysis

The results were given as mean ± standard deviation (SD), statistical evaluation of the results was performed by one-way ANOVA along with the level of significance set at $P < 0.05$ and the Chi-Square test for mortality.

RESULTS

The body weight, growth rate, feed intake, feed conversion rate, carcass yield and mortality of rabbits during the experiment are presented in table 2. The digestible energies of Control diet C and feed FR are iso-protein, and iso-energetic are very low compared to those recommended by Lebas (2004).

The weight of animals fed FR tended to be somehow higher than that of animals fed Control diet. The FR group consumed a little more than the C group, especially after the 2nd week of treatment. There was no significant difference

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in the growth rate of the animals given the FR diet or the animals given the Control diet. The feed conversion rate is similar in the two groups during the 21 days of treatment. The carcass yield and livers of rabbits did not change significantly with the addition of the 2.5% combination of fennel and rosemary in their feed. Moreover, the digestive disorders that characterize the weaning period manifested by severe diarrhea and put animal health in danger. Mortality in the FR group was better than that recorded in the control group C.

Effects of dietary fennel and/or thyme on caecal microbial counts are presented in Table 3. There was no significant difference in the number of *E.coli* in the cecum of rabbits fed control diet or FR diet. The chemical composition of the essential oils of *Foeniculum vulgare* and *Rosmarinus officinalis* are shown in Table 4. The essential oil of *Foeniculum vulgare* seeds is dominated by the trans anethole compound and by fenchone. The fundamental component of the essential oil of *Rosmarinus officinalis* is 1-8 cineol, together with α-pinene, the α-phellandrene, and camphene.

The results of the antibacterial activity of essential oils against *E. coli* are shown in Table 5. The essential oil of the rosemary tested did not show an *E.coli* antibacterial effect. The combination of the essential oil also did not demonstrate antibacterial power against these bacteria. This gives reason for the low impact of these phytobiotics on *E.coli* bacteria in the cecum. The addition of the 2.5% combination of fennel and rosemary in rabbit feed did not influence the zootechnical parameters of the rabbits.

Oxytetracycline and *Rosmarinus officinalis* essential oil did not have activity against *E. coli*. The essential oil of seeds of the *Foeniculum vulgare* showed low activity against the bacteria with 1.4 cm zone of inhibition. The combination of the two essential oils *Foeniculum vulgare* and *Rosmarinus officinalis* was to be ineffective against *E. coli*.

The minimum inhibitory concentrations (MIC) and bactericidal (MBC) of essential oils against *E. coli* are grouped in table 6. The essential oil of *Foeniculum vulgare* showed the lowest antibacterial effect (MIC = 50% v/v) against *E.coli* with no bactericidal action at the concentrations tested. The essential oil of *Rosmarinus officinalis* was inactive concerning the strain tested in this study (MIC > 50 M L/ml).

Table 2 - Effect of dietary supplementation with fennel seeds and rosemary leaves on rabbit growth performance and mortality

Traits \ Group (mean ± SD)	Days	Control diet	Fennel and Rosemary diet	P value
Body weight (BW), g	40	880±80	916±130	0.217
	47	1143±100	1164±170	0.598
	54	1321±165	1414±200	0.162
	61	1501±190	1619±209	0.152
	71	1821±159	1943±207	0.191
Feed intake, g. d–1	40	81.4±7	76.5±6	0.059
	47	97.5±12	99±7	0.666
	54	101.7±11	108.4±19	0.271
	61	134±9	138±10	0.647
	71	134.2±14	137.7±13	0.649
Growth rate, g. d–1	40-47	39.1±13	37±13	0.560
	47-54	42.9±32	35.2±9	0.399
	54-61	35.6±10	38.6±6	0.402
	61-71	37.2±10	33.9±6	0.413
Feed conversion ratio	40-47	2.36±0.7	2.49±0.9	0.588
	47-54	3.14±0.9	3.22±0.6	0.797
	54-61	3.36±0.9	3.33±0.5	0.082
	61-71	3.74±0.8	4.1±0.7	0.409
Carcass yield, (% of BW)	50	56.4±3	51.4±6	0.294
	60	55.6±5	52.9±3	0.445
	71	54.4±1	52.9±1	0.114
Liver yield, (% of BW)	50	3.7±0.4	3.8±0.3	0.672
	60	3.6±1	4.1±1	0.584
	71	4 ±0.1	4±0.2	0.605
Mortality [1] ,%	40-71	42.9	46.4	> 0.05

[1] mortality are analysed using a χ 2 test at P < 0.05

Table 3 - Counts of *E. coli* in caecum of rabbits (log 10 cfu . g–1; mean ± SD)

Days	Bacteria	Group		P value
		Control diet	Fennel and Rosemary diet	
40	*E.coli*	3.3 ±0.3		
50	*E.coli*	3.94±0.2	4.35±0.3	> 0.05
60	*E.coli*	3.93±0.3	3.79±0.3	> 0.05

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Table 4 - Chemical composition of essential oil			
Component	**Retention time (min)**	***Foeniculum vulgare* seeds**	***Rosmarinus officinalis***
Myrcene	6.55		
Para cymene	7.19		
cis-Ocimene	7.73	0.41	
α-Pinene	7.75		
γ terpinne	7.79		
α-Pinene	8.28		18.94
Linalol	8.51		
Camphene	8.74		5.38
α-Phellandrene	9.70		5.46
Terpinène -4-ol	9.79		
α-Pinene	11.01	2.22	
dl-Limonene	11.01		
Thymol	11.38		
Carvacrol	11.55		
1-8 cineol	11.68		51.62
Fenchone	12.99	6.14	
Fenchone	13.00		
β-Caryophyllene	13.08		
α–Campholene aldehyde	15.63		10.65
Borneol	16.47		2.59
Isopulegyl acetate	17.81		
α-Fenchyl acetate	18.23		
Trans Anethole	20.07	91.12	
Trans Anethole	20.10		

Table 5 - Diameter of inhibition zones of essential oil against *E.coli* (cm)				
Bacterial strain	**Antibiotic**	**Essential oil**		
	Oxytetracycline	***Foeniculum vulgare***	***Rosmarinus officinalis***	***Foeniculum vulgare* +*Rosmarinus officinali***
E.coli	0.6±0	1.4±0.6	0.6±0	0.9±0.5

Table 6 - Minimum inhibitory concentration, minimum bactericidal concentration en µl/ml.				
Bacterial strain		**Essential oil**		
		Foeniculum vulgare	***Rosmarinus officinalis***	***Foeniculum vulgare* + *Rosmarinus officinalis***
E.coli	CMI	50	>50	>50
	CMB	>50	>50	>50

DISCUSSION

The efficacy of combination of fennel and rosemary in rabbit feed was not showed in this work. Several studies have demonstrated positive effects of phytobiotics as feed additives in the diet of animals. Omer et al. (2013) found that adding 0.5% fennel seed with 0.5% oregano leaves as feed additives improved performance parameters of rabbits. Ghazalah and Ali (2008) results confirm the significant improvement in weight as well as in daily weight gain in chicks fed a feed supplemented with 0.5% leaves of *Rosmarinus officinalis*. They noticed that with this dose the zootechnical parameters are better than higher doses (1.2%). Likewise, the study by Al kassi, (2008) showed this positive effect of daily weight gain but with the use of 1% of *Rosmarinus officinalis* in chicken feed. Abdullah et al. (2009) also observed that supplementation of broiler diets with 1, 2 and 3 g/Kg of fennel seeds improved weight gain. Cesari et al. (2008) found a significant improvement in rabbit weight, daily weight gain, and conversion index and the use of the combination of organic acids and essential oils from rosemary, thyme, and cinnamon. Mathlouthi et al. (2012) noticed these positive effects with the addition of 50 mg/Kg in chicken feed. However, Erdelyi et al. (2008) did not find a positive or a significant impact on these zootechnical parameters of rabbits within the use of essential oils from rosemary and garlic. The mortality performance results were close to the results obtained by Erdelyi et al. (2008) as 48% that incorporated 0.15% of the essential oil of *Rosmarinus officinalis* in the diet of rabbits. However, Benlemlih et al. (2020) demonstrated that fennel dietary supplements (5%) on the feeding of rabbits decreases mortality. According to Cesari et al. (2008)'s study, the mortality showed to be reduced to 42% compared to the control group within the use of a combination of organic

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acids and rosemary's essential oil, thyme, and cinnamon. Fotea et al. (2009) noticed that the mortality of chickens decreased with the 0.5% dose, better than with 1 and 1.5% rosemary added to their feed. The lower dosage used in this experiment with the low antibacterial activity of the combination of the essential oil of *Rosmarinus officinalis* and *Foeniculum vulgare*, did not prevent the breeding of the high mortality rate. Moreover, the essential oil combination also did not demonstrate antibacterial power against *E.coli*. which gives reason for the low impact of these phytobiotics on this bacterium in the cecum. However, reduced counts of *E. coli* was showed by Benlemlih et al. (2014) during the application of dried fennel and thyme to rabbit.

Following our results, the essential oil yield of the *Foeniculum vulgare* (2.11 %) and *Rosmarinus officinalis* (0,49%) was similar to values published by Stefanini et al. (2006) for the Brazilian fennel (2.7%) and by Moujahed et al. (2011) for the Tunisian rosemary (0.43%). This study showed that the essential oils of *Foeniculum vulgare* are distinguished by a large percentage of the phenol phenylpropanoids. The essential oil of *Rosmarinus officinalis* consists of terpenes and oxygen-containing terpenes. The low antibacterial activity recorded by essential oils of seeds and leaves of *Foeniculum vulgare* has already been demonstrated by the study of Grigore et al. (2012). The essential oil of *Rosmarinus officinalis* was ineffective against our isolated bacterium despite its capacity in oxygenated monoterpenes (51%). Ait-Ouazzou et al. (2012) studied the antimicrobial effect of 11 major constituents of essential oils and found that the 1.8 cineole had moderated activity compared with other oxygenated monoterpèniques compounds such as carvacrol, thymol, and, linalool.

The yield of rosemary essential oil is very low. In our phytobiotic the small content of essential oil contained only (0.038%) within the addition to its low antibacterial activity which may explain its negativity on mortality and its power on growth and other parameters.

CONCLUSION

The addition of the 2.5% combination of fennel and rosemary in rabbit feed did not influence the zootechnical parameters of the rabbits. The presence of Trans anethole in fennel essential oil involves in reduction of the number of *E. coli* suggested that other doses of fennel as feed additives should be studied.

DECLARATIONS

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Authors' contribution
All authors contributed to research conduction, analyzing and writing equally.

Conflict of interests
The authors declare that there is no conflict of interests in this work.

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