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Effect of Dietary Dried Fennel and Oregano and Thyme Supplementation on Zootechnical Parameters of Growing Rabbits

Majda Benlemlih*, Amina Barchan, Ahmed Aarab, Mohammed Bakkali, Abdelhay Arakrak, and Amin Laglaoui

Department of Biology Faculty of Science and Technology, Abdelmalek Essaâdi University 416 Tangier, Morocco

*Corresponding author`s Email: benlemlihmajda@yahoo.fr; ORCID: 0000-0002-2704-5140

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ABSTRACT

The objective of this study was to analyze and compare the effects of fennel, oregano, and thyme dietary supplements on the feeding of rabbits. In this regard, 96 weaned rabbits (30-day-old), white New Zealand, were divided into 4 groups and submitted to the following dietary treatments: Control diet, F diet (Control diet + 5% *Foeniculum vulgaris*), O diet (Control diet + 5% Origanum compactum), and T diet (Control diet + 5% *Thymus capitatus*). The essential oils of the above mentioned aromatic plants were extracted and were analyzed using a gas chromatograph coupled to a mass spectrometer. The treatment of fennel, oregano, and thyme had no beneficial effects on the growth performance of the rabbits but reduced the mortality rate. The phenylpropanoid and the phenolic monoterpenes were the major components of *Foeniculum vulgaris*, *Origanum compactum*, *and Thymus capitatus* essential oils. The aromatic plants and their active compounds can be used as additives in rabbit nutrition.

Keywords: Essential oil, Fennel, Growth performance, Oregano, Rabbit, Thyme

INTRODUCTION

The ban on antibiotics as growth promoters in feed by the European Commission (EC) in January 2006 due to cross and multiple bacterial resistances (Huyghebaert et al., 2011) has led to the search of new practices to decrease rabbit health problems. The use of aromatic plants to reduce the rate of health incidents and death from diarrhea and to increase production efficiency was one alternative additive tried in growing rabbits (Efterpi et al., 2012).

Foeniculum vulgare was used for bacterial, fungal and viral infections. It has anticolitic activity and was indicated in the treatment of stomatitis, spastic gastrointestinal disturbances, abdominal cramps and flatulence (Badgujar et al., 2014; Al-Snafi, 2018). *Origanum compactum* has been traditionally used to treat diarrhea (Ennabili et al., 2004) and *Thymus capitatus*is widely used as a stomachic (Megdiche-ksouri et al., 2015). The antibacterial and antifungal activities of the essential oils of oregano and thyme were established by several studies (Bounatirou et al., 2007; Bouhdid et al., 2009; El Ouariachi et al., 2011; Bouyahya et al., 2016; El Jalel et al., 2018). The aim of this study is to examine the effects of *Feoniculum vulgaris and Origanum compactum* and *Thymus capitatus*as natural feed supplements on growth performance in weaned rabbits.

MATERIAL AND METHODS

Medicinal plants

The areal parts of tree aromatic and medicinal plants *Foeniculum vulgaris*, *Origanum compactum* and *Thymus capitatus* were collected in northern Morocco. Identification of Aromatic plants was executed by Professor Bakkali, a specialist in botany, in the Laboratory of Biotechnology and Biomolecular Engineering. Afterwards, the leaves were separated and dried at room temperature for two weeks in the absence of light and then stored in sealed paper bags until their use for analyses.

Animals and experimental procedure

A total of 96 weaned rabbits (30 days old; white New Zealand (900 ± 100 g initial weight), were divided into four groups and submitted to the following dietary treatments (Table 1). The first group was control diet, the second group was F diet (Control diet + 5% *Foeniculum vulgaris* leaves), the third group was O diet (Control diet + 5% *Origanum compactum* leaves) and the last group was T diet (Control diet + 5% *Thymus capitatus* leaves). Rabbits were kept in standard cages with 6 animals per cage (2010/63/EU Official Journal of the EU 2010) in a building with temperatures between 15 and 20 °C. The length of daily illumination was 16 hours. The rabbits had access to feed and water *ad libitum*. Body weight of rabbits and feed consumptions were measured every week during the experiment as well as mortality rates.

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Table 1. Ingredients and chemical composition and nutritive value of diets for male and female white New Zealand rabbits

Ingredients	(%)	Chemical composition	Control diet	Fennel diet	Oregano diet	Thyme diet
			(g/100g)	(g/100g)	(g/100g)	(g/100g)
Bran	28.5	Dry matter	89.9	90.02	89.7	89.7
Corn	9.5	Ash	7.7	8.22	7.9	7.3
Soy bean meal	9.5	Crude protein	20.3	20.5	19.9	19.7
Sun flower meal	14.2	Ether extract	5.8	5.6	5.8	6.1
Alfalfa	33.75	NDF	30.5	29.9	30.7	30.6
Vegetable oil	2.8	ADF	17.5	17,5	18.4	18.1
Premix[1]	0.6	ADL	4.6	4.5	5.1	4.7
Salt	0.5	**Digestible energy** (Kcal/Kg)	2522	2549	2502	2528
DL Methionine	0.1					
L-Lysine	0.2					
Dicalcium phosphate	0.25					
Calcium carbonate	0.1					

[1] 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, 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, 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 chloride and QSP calcium. Premix contained 50 ppm of Salinomycin; NDF: neutral detergent fibre; ADF: aciddetergent fibre; ADL: detergentlignin.

Chemical analysis

Chemical analyses of diets were 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 values of diets. The essential oils were extracted via steam distillation for 2 hours using a Clevenger-type apparatus. The supernatant was separated by decantation after adding 50% NaCl. The essential oils were stored in sealed glass vials at 4◦C prior to analysis. Gas chromatography–mass spectrometry (GC-MS) analysis was performed using a gas chromatograph (Trace GC ULTRA; Thermo Scientific, Waltham, MA, USA) coupled to a mass spectrometer (Polaris Q MS with ion trap; Thermo Scientific) in the electron impact (EI) ionisation mode (70 eV) in the 50–350 m/z range. The analysis was carried out using a VB-5 methyl polysiloxane at 5% phenyl) (Thermo Scientific) column (30m × 0.25mm, film thickness 0.25 µ m) using a temperature program of 40–300◦ Catarate4◦C min−1. Injector temperature was set at 220◦C. Helium gas was used as the carrier gas at a constant flow rate of 1.4 mL min−1. Diluted samples (1% in n - hexane; Sigma–Aldrich, Steinheim, Germany) of 1.0 µL were injected in the split mode to allow better identification of compounds. The analysis was repeated twice for each sample. The constituents were identified by comparison of their retention indices and mass spectra with those in the computer library (NIST MS Library Search, v.6.0) and with literature data.

Statistical analysis

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

RESULTS

Live weight, growth rate, feed intake, feed conversion rate and the mortality of rabbits during the experiment are presented in table 2. In general, no significantly differences in feed intake or feed conversion were observed in rabbits fed with different diets but the group of rabbits fed with the fennel supplemented diet appeared to eat bigger quantities of feed and presented a higher body weight when compared to the control group of rabbits at two weeks after weaning. The body weight varies significantly ($P<0.05$) at the age of slaughter and the thyme group presented the best body weight and the higher growth rate in the last week of experience. Mortality rate was significantly ($P<0.05$) lower for rabbits fed with fennel or oregano or thyme supplemented diets when compared to those fed with the control diet during growing period.

Essential oils yields expressed in relation to dry weight plant material are shown in table 3. The yield of the essential oils, based on the dry weight of the samples, was 1.89%, 2.92% and 1.96 % for *Foeniculum vulgaris*, *Origanum compactum* and *Thymus capitatus* respectively. The yield of essential oil of *Origanum compactum* (around 3%) was higher than the other aromatic plants. The chemical composition of essential oils of *Foeniculum vulgaris*, *Origanum compactum* and *Thymus capitatus* is presented in table 3. The essential oils of *Foeniculum vulgaris* was dominated by

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anethol (83.29%) and followed by limonene (14.40%). The carvacrol (68.99%) was the main compound in *Origanum compactum* oil followed by thymol (18.67 %). The *Thymus capitatus* oil was dominated by carvacrol too (95.25%).

Table 2. Effects of dietary supplementation of fennel oregano and thyme in growth performance and mortality of 30 days old white New Zealand rabbit

Indices	Days	Group				
		Control	Fennel	Oregano	Thyme	P value
Body weight (g) ± SD	30	580±60 [a]	570±90[a]	570±47[a]	600±79[a]	0.146
	37	800.3±165[a]	911.4±218[a]	651.1±139[a]	711±72[a]	0.176
	44	920±156[a]	1080±155[a]	850±92[a]	981.3±80[a]	0.118
	51	1300.5±74[a]	1206±82[a]	1129.3±117[a]	1 220±106[a]	0.152
	58	1750±37[b]	1438.8±91[a]	1 460±101[a]	1 630±68[b]	< 0.001
	70	2209±103[bc]	1979±155[a]	2068.6±72[ab]	2304.9±28[c]	0.002
Feed intake (g .d^{-1}) ± SD	30	47.9±4[b]	30.4±4[a]	35,4±10[a]	30.4±4[a]	0.006
	37	50±14[a]	62.5±20[a]	72.2±25[a]	36.7±4[a]	0.075
	44	66.6±10[a]	76.5±12[a]	65±10[a]	68.9±5[a]	0.422
	51	91.9±6[a]	91.6±6[a]	90,3±10[a]	78.6±9[a]	0.095
	58	107.5±22[a]	92.6±6[a]	106.7±8[a]	108.3±12[a]	0.374
	70	108.1±9[b]	93.8±6[a]	112.0±8[b]	112.5±6[b]	0.013
Growth rate (g .d^{-1}) ± SD	30-37	37.5±2[a]	45.4±33[a]	14.7±19[a]	14.8±8[a]	0.167
	37-44	15.7±4[a]	31.0±13[a]	32.9±7[a]	35.5±7[a]	0.063
	44-51	49.0±19[b]	18.1±13[a]	39.9±4[b]	34.1±4[ab]	0.022
	51-58	64.2±6[c]	33.1±5[a]	47.3±3[b]	58.6±6[c]	< 0.001
	58-70	38.3±5[a]	45.1±4[a]	50.7±2[bc]	54.0±3[c]	< 0.001
Feed conversion ratio ± SD	30-37	2.41±0.7[a]	2.56±0.7[a]	2.45±1[a]	2.23±1.1[a]	0.983
	37-44	3.19±0.5[a]	3.18±2[a]	2.29±1.2[a]	1.80±0.2[a]	0.788
	44-51	2.36±0.4[a]	2.83±1[a]	2.04±0,1[a]	2.03±0,2[a]	0.383
	51-58	2.60±0.5[ab]	3.75±0.2[c]	3.26±0.5[bc]	2.41±0.3[a]	0.028
	58-70	2.96±0.4[b]	2.05±0.2[a]	2.13±0.2[a]	1.96±0.1[a]	0.002
Mortality (%) ± SD	30 -58	63.63[b]	33.33[a]	41.6[a]	41.6[a]	< 0.05
	30 -70	68.18[b]	37.5[a]	41.6[a]	41.6[a]	

SD: Standard deviation; Mortality ratesare analysed using a $\chi 2$ test; The values with different superscript letters in a row are significantly different ($p<0.05$).

Table 3. Chemical composition of essential oils of *Foeniculum vulgaris, Origanum compactum* and *Thymus capitatus*

Component \ Concentration			Peak area %	
	Retention Time (min)	Foeniculum Vulgaris (%)	Origanum Compactum (%)	Thymus Capitatus (%)
Myrcène	6.55	-	0.44	-
Para cymene	7.19	-	2.53	-
cis-Ocimene	7.73	-	-	-
α-Pinene, (-)-	7.75	0.12	-	-
γtérpinene	7.79	-	3.98	-
α-Pinene, (-)-	8.28	-	-	-
Linalol	8.51	-	1.09	-
Camphene	8.74	-	-	-
α-Phellandrene	9.70	-	-	-
Terpinene -4-ol	9.79	-	0.58	-
α-Pinene, (-)-	11.01	-	-	-
dl-Limonene	11.01	14.40	-	-
Thymol	11.38	-	18.67	-
Carvacrol	11.55	-	68.99	-
1-8 cineol	11.68	-	-	-
Fenchone	12.99	-	-	-

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Fenchone	13.00	1.32	-	-
β-Caryophyllene	13.08	-	1.08	-
α -Campholene Aldehyde	15.63	-	-	-
Borneol	16.47	-	-	-
Isopulegyl acetate	17.81	0.12	-	-
α-Fenchylacetate	18.23	0.76	-	-
Trans Anethol	20.07	-	-	-
Trans Anethol	20.10	83.29	-	-
Carvacrol	20.69	-	-	95.25
Caryophyllene	24.24	-	-	1.49
Caryophyllene	24.89	-	-	-
Tetradecamethylcycloheptasiloxane	27.26	-	-	-
3,5-Diethylphenol	37.95	-	-	0.74
1,15-Dihydrohexadecamethyloctasiloxane	38.85	-	-	0.91
6-Acetyl-2,2-dimethyl-8-(3-methyl-2-butenyl)-2H-1-benzopyran	39.37	-	-	-

DISCUSSION

Weaning of the young rabbits is the most critical period; it influences their health, their growth and mortality rate. Aromatic plants have been found to possess many biological activities with their natural compounds that why they are demanded in food.

Omer et al. (2013) found that adding 0.5% fennel seed with 0.5% oregano leaves as feed additives improved performance parameters of rabbits. Abdullah et al. (2009) also observed that supplementation of broiler diets with 1, 2 and 3 g/Kg of fennel seeds improved weight gain. These findings were according to present experimental results about 5% of fennel. This aromatic plant helps to improve the appetite with the anethole and limonene, active compound in the essential oil of present *Feniculum vulgaris.* Anethole has digestive stimulating and appetizing effects (Cabuk et al., 2003). That could explain the bigger quantity consumed and the best weight gain by this group in the first weeks. Badgujar et al. (2014) reported that essential oil of fennel reduces intestinal gas and regulates the motility of smooth muscles.

According to Ayala et al. (2011) dietary supplementation with dried oregano improved rabbit performance. Cardinali et al. (2015) showed that supplementation with 0.2% oregano can improve productive performance and carcass quality in rabbits. Giannenas et al. (2005) also found that dehydrated oregano plants (5g/kg) exerted a growth promoting effect when incorporated in chicken diets. However, other researchers found that dietary oregano essential oil exerted no growth-promoting effect on rabbits (Botsoglou et al., 2004). The dietary inclusion of dried oregano (10 or 20 g/kg diet) had also no effect on the performance parameters of laying quail (Christaki et al., 2012). These results are in agreement with present observations that indicated oregano did not positively affect body weight gain and feed intake in rabbit. Oregano had higher essential oil yield than thyme; this last aromatic plant did not affect performance parameters of rabbit but body weight was better at age of slaughter.

Contradictory results on the effects of thyme or its essential oil are reported for rabbits. In some cases, growth performance improved (Dalle Zotte et al., 2014; Gerencser et al., 2014; Abdel-Wareth et al., 2018), but in others there was no effect (Soultos et al., 2009; Gerencser et al., 2012). Ocak et al. (2008) indicated also that thyme did not significantly affect the growth performance of broilers.

The crude protein and digestible energies of all dietary treatments were in the superior range recommended for growing rabbits, which contributed to high rate mortality. Present results showed that supplementing the diet with fennel or oregano or thyme reduced the mortality rate approximately 40% compared to the control diet. Low mortality rate of rabbit repoted by using sage and oregano extracts (Szaboova, 2007). The treatment with fennel seeds and thyme or their essential oil had also a beneficial effect on the mortality rate (Benlemlih et al., 2014). There is an evidence to suggest that aromatic plant with components of the essential oil may have a role in combating bacterial diseases in rabbit and reducing mortality. Placha et al. (2013) observed that dietary inclusion of 0.5 g/kg of thyme essential oil was able to limit the colonization of coliforms in the caecum. Reduced counts of coliforms and clostridia, *E. coli* and *S. aureus* was presented by Benlemlih et al. (2014) during the application of dried fennel and thyme to rabbit. The essential oil of present aromatic plants contains a high percentage of phenolic compounds such as carvacrol and thymol, the most important antibacterial properties (Sokovic, 2010). Koné et al. (2016) observed a small positive effect of polyphenols in reducing bacterial microflora of rabbit meat. It was also mentioned that thymol possess antibacterial effect against various gram positive and negative bacterial strains (Meeran et al., 2017). Anethole and limonene present in fennel oil are responsible for its antimicrobial effects (Gulfraz et al., 2008; Vimal et al., 2013). The antispasmodic activity has also

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been shown for thymol and carvacrol (Keyhanmanesh and Boskabady, 2012). There are some evidences that major components of present aromatic plants have a different biological activity and have potential health effects in rabbit lifespan.

CONCLUSION

It could be concluded that supplementing rabbit diets with 5% of thyme has a significant beneficial effect on performance. Fennel, oregano and thyme had potential health effects and reduced mortality rates. Aromatic plants and their active compounds can be tried as additives substances for rabbit nutrition and improving health performance.

DECLARATIONS

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Competing interests

The authors declare that they have no competing interests.

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