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# The effect of supplementing diets with dried fennel and thyme on the zootechnical parameters and caecal microflora of growing rabbit

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**ABSTRACT.** The objective of this study was to evaluate the effect of fennel and thyme dietary supplements on the feeding of rabbits. Eighty-five weaned rabbits (35 days old), white New Zealand (of both sexes), were divided into four groups and submitted to the following dietary treatments: Control diet, diet F (Control diet + 2.5% *Foeniculum vulgare* seeds), diet T (Control diet + 2.5% *Thymus capitatus* leaves) and diet FT (Control diet + 2.5% *Foeniculum vulgare* seeds and *Thymus capitatus* leaves) for twenty-five days. The treatment with fennel and thyme had a beneficial effect on the mortality rate (18%). However the growth rate, feed conversion ratio and carcass yield were not influenced by dietary fennel and/or thyme supplementation. The antimicrobial effect of thyme (2.5%) was observed only against *C. perfringens* in the caecum ($P < 0.05$), but no effect was observed on the caecal count of or *C. perfringens* in the other treated groups.

## Introduction

Weaning is the most critical period in rabbit breeding; it is associated with a higher risk of digestive disorders in growing rabbits (Krieg et al., 2009). Antibiotics as growth promoters (AGPs) have been widely used in specific diets to prevent these digestive disorders. Recently, the concerns about possible antibiotic residues and disease resistance have aroused great caution in their use in the animal industry (Jang et al., 2007). In January 2006, the European Commission banned the marketing and use of antibiotics as growth promoters in feed (Huyghebaert et al., 2011). The decreased use of AGPs in animal diets necessitates finding alternative feed additives. Aromatic plants are being explored as options to antimicrobials, due to their different active substances. The influence of some medicinal plants and their extracts on rabbit has been studied (Eiben et al., 2004; El-Nattat and El-Kady, 2007; Soultos et al., 2009; Simonová et al., 2010; Gerencsér et al., 2012). Krieg et al. (2009) found that a herbal feed additive had a positive influence on performance and health in weaned rabbits.

Fennel (*Foeniculum vulgare*) and its preparations are used to cure various disorders, and also act as a carminative, digestive and diuretic agent. Anethole and its isomers present in fennel oil are responsible for its antimicrobial effects (Gulfraz et al., 2008). Several studies have been published on the biological properties of *Thymus capitatus* as an antibacterial, antifungal and antioxidant agent (El Ouariachi et al., 2011).

The aim of this study was to examine the effects of *Foeniculum vulgare* and *Thymus capitatus* as natural feed supplements on growth performance and caecal microflora in weaned rabbits.

## Material and methods

### Animals and experimental procedure

A total of 85 weaned rabbits white New Zealand (35 days old; male and female; 800 ± 100 g initial body weight), were divided into four groups and submitted to the following dietary treatments (Table 1) for twenty-five days: Control diet; diet T (2.5% *Thymus capitatus* leaf powder mixed with the Control diet); diet F (2.5% *Foeniculum vulgare* fruit powder mixed with the Control diet) and diet FT (2.5% *Thymus capitatus* and *Foeniculum vulgare* powder mixed with the Control diet).

The rabbits were kept in standard cages (7 animals per cage, except the last FT cage with 8 animals) 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 body weight of rabbits, feed consumption and mortality rates were measured every week during the experiment.

### Chemical analysis

Chemical analysis of diets was performed following the methods of AOAC (1991) for dry matter, ash and crude protein, and of Van Soest et al. (1991) for acid detergent fibre (ADF), neutral detergent fibre (NDF) and detergent lignin (ADL).

**Table 1**. Ingredients and chemical composition and nutritive value of diets

| Ingredients | % | Chemical composition, g · 100 g$^{-1}$ | |
|---|---|---|---|
| Wheat bran | 28.5 | Dry matter | 92.2 |
| Maize | 9.5 | Ash | 8.71 |
| Soyabean meal | 9.5 | Crude protein | 21.1 |
| Sunflower meal | 14.2 | Ether extract | 5.28 |
| Lucerne, hopped | 33.75 | NDF | 39.4 |
| Vegetable oil | 2.8 | ADF | 20.0 |
| Salt | 0.5 | ADL | 4.52 |
| Premix[1] | 0.6 | Digestible energy, kcal · kg$^{-1}$ | 2522 |
| DL-Methionine | 0.1 | | |
| L-Lysine | 0.2 | | |
| Dicalcium phosphate | 0.25 | | |
| Calcium carbonate | 0.1 | | |

[1] 1 kg of Premix provides: IU: vit. A 1000000, vit. D 300000; g: vit. E 2, vit. K 0.4, vit. $B_1$ 0.075, vit $B_2$ 0.4, vit. $B_3$ 1.218, vit. $B_5$ 0.099, vit $B_6$ 0.083, vit. $B_9$ 0.190, vit. $B_{12}$ 0.030, biotin 0.005, cuivre 0.2, Fe 4, Zn 5, I 0.012, Se 0.012, Co 0.020, Mn 6, choline chloride and QSP calcium 57; Premix contained 50 ppm of salinomycin

### Bacteriological analysis

Three animals from each group were slaughtered on days 35, 45 and 60. Bacteria from caecal samples were isolated by the standard microbiological method using the appropriate dilutions in Ringer solution. Dilutions were plated onto the following media: Mac Conkey agar for *E. coli,* incubated at 37°C for 24 h and Tryptose Sulphite agar with the antibiotic, D-Cycloserine (TSC) for *C. perfringens*, incubated for 48 h at 37°C, with the counts determined according to the ISO 7937 standard (1997). The bacterial counts were expressed in colony forming units per gram (log10 CFU · g$^{-1}$).

### Statistical analysis

The results are given 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 the Chi Square test for mortality.

## Results

The liveweight, growth rate, feed intake, feed conversion rate, carcass yield and mortality of rabbits during the experiment are presented in Table 2.

The group of rabbits fed with fennel- and/or thyme-supplemented diet appeared to eat large quantities of feed and presented a higher body weight and growth rate when compared with the control group during the first week of treatment. These parameters were not influenced by dietary fennel or thyme supplementation after this period.

Although the feed conversion rate did not differ significantly ($P < 0.05$) when the experimental period was considered as a whole, the rate of conversion for groups F and T tended to be better during the third week of treatment. The mean carcass yield also did not differ significantly among the groups, but the liver yield varied significantly only after ten days of treatment. The highest mortality rate was observed in the control group, followed by the F and then the T group, with the FT group presenting the lowest mortality.

Effects of dietary fennel and/or thyme on caecal microbial counts are presented in Table 3. The CFU of in digesta taken from the caecum were not influenced by dietary supplementation of medicinal plants. However, rabbits fed diets containing thyme (2.5%) showed a significant ($P < 0.05$) reduction in CFU of *C. perfringens* after three weeks of treatment (on day 60) when compared with the others.

**Table 2.** Effect of dietary supplementation with fennel seeds and thyme leaves on rabbit growth performance and mortality

| Indices | Days | Group | | | | |
|---|---|---|---|---|---|---|
| | | C | F | T | FT | *P* |
| Body weight (BW), g; mean ± SD | 35 | 782 ± 80 | 811 ± 94 | 802 ± 88 | 796 ± 80 | 0.74 |
| | 42 | 977 ± 111 | 1048 ± 120 | 1093 ± 130 | 1070 ± 120 | 0.047 |
| | 49 | 1143 ± 158 | 1195 ± 180 | 1253 ± 180 | 1278 ± 173 | 0.227 |
| | 56 | 1363 ± 250 | 1358 ± 200 | 1488 ± 135 | 1386 ± 174 | 0.434 |
| Feed intake, g · d$^{-1}$; mean ±SD | 35 | 53.7 ± 10 | 59.7 ± 12 | 66.0 ± 10 | 61.0 ± 8 | 0.093 |
| | 42 | 63.0 ± 13 | 77.7 ± 7 | 88.3 ± 10 | 106.7 ± 11 | 0.006 |
| | 49 | 97.0 ± 15 | 101.3 ± 9 | 100.3 ± 10 | 110.6 ± 11 | 0.591 |
| | 56 | 136.5 ± 19 | 108.3 ± 20 | 113.7 ± 17 | 116.6 ± 22 | 0.395 |
| Growth rate, g · d$^{-1}$; mean ± SD | 35–42 | 28.4 ± 13 | 33.9 ± 10 | 38.7 ± 15 | 39.0 ± 11 | 0.03 |
| | 42–49 | 26.6 ± 9 | 32.0 ± 13 | 31.5 ± 18 | 31.9 ± 13 | 0.713 |
| | 49–56 | 32.9 ± 14 | 21.9 ± 9 | 24.6 ± 20 | 16.0 ± 8 | 0.388 |
| Feed conversion ratio; mean ± SD | 35–42 | 2.24 ± 0.7 | 2.25 ± 0.6 | 2.08 ± 0.8 | 2.25 ± 0.7 | 0.91 |
| | 42–49 | 2.99 ± 0.7 | 3.51 ± 0.9 | 2.80 ± 0.8 | 3.33 ± 0.9 | 0.947 |
| | 49–56 | 4.18 ± 0.9 | 3.42 ± 0.6 | 3.05 ± 0.8 | 5.90 ± 0.4 | 0.006 |
| Carcass yield, (% of BW) mean ± SD | 45 | 51 ± 4 | 48 ± 2 | 47 ± 6 | 49 ± 1 | 0.773 |
| | 60 | 47 ± 9 | 52 ± 1 | 53 ± 2 | 58 ± 10 | 0.425 |
| Liver yield, (% of BW); mean ± SD | 45 | 3.0 ± 0.5 | 4.3 ± 0.4 | 3.7 ± 0.4 | 5.1 ± 0.7 | 0.01 |
| | 60[d] | 4.0 ± 0.7 | 4.6 ± 0.4 | 4.1 ± 1 | 5.3 ± 0.7 | 0.406 |
| Mortality[1],%; mean ± SD | 35–42 | 4.8 | 0 | 0 | 0 | |
| | 42–49 | 14.3 | 4.8 | 19.0 | 0 | |
| | 49–56 | 52.4 | 33.3 | 9.5 | 18.1 | |
| | 35–60 | 71.4 | 38.1 | 28.6 | 18.1 | <0.05 |

[1] mortality are analysed using a $\chi$ 2 test at $P < 0.05$

**Table 3**. Counts of *E. coli* and *C. perfringens* in caecum of rabbits (log 10 cfu · g$^{-1}$ (mean ± SD)

| Days | Bacteria | Group | | | | |
|---|---|---|---|---|---|---|
| | | C | F | T | FT | *P* |
| 35 | *E .coli* | | 2.98 ± 0.5 | | | |
| | *C. perfringens* | | 2.81 ± 0.5 | | | |
| 45 | *E. coli* | 4.03 ± 0.6 | 3.99 ± 0.1 | 3.59 ± 0.8 | 3.80 ± 0.4 | 0.814 |
| | *C. perfringens* | 2.92 ± 0.3 | 2.79 ± 0.1 | 2.82 ± 0.3 | 2.75 ± 0.3 | 0.895 |
| 60 | *E. coli* | 4.56 ± 0.2 | 4.41 ± 0.1 | 3.94 ± 0.6 | 4.09 ± 0.6 | 0.606 |
| | *C. perfringens* | 3.04 ± 0.1 | 2.60 ± 0.2 | 1.69 ± 0.1 | 2.50 ± 0.4 | 0.035 |

# Discussion

In recent years, aromatic plants and their extracts have received increased attention as potential alternatives to antibiotic growth promoters. It is known that their pharmaceutical properties are partially due to the essential oils they contain (Edris, 2007). There is very limited information on the effects of herbs or their compounds on the growth of rabbits. In agreement with our present study, Gerencsér et al. (2012) found that supplementing the diet with 3% thyme had no effect on rabbit weight gain or feed consumption. Those parameters were also not affected by supplementation with dietary oregano essential oil (Soultos et al., 2009). Sengül et al. (2008) observed no change in weight gain or feed conversion when growing Japanese quails were fed diets supplemented with 2.5 ml · kg$^{-1}$ thyme oil or 100 ml · l$^{-1}$ extracted water of thyme (*Thymus vulgaris* L.). Hernandez et al. (2004) found that the addition of 5,000 ppm *Labiatae* extract from thyme, sage and rosemary to broilers diets had no beneficial effect on the feed conversion ratio or on feed intake. Bampidis et al. (2005) also observed that supplementation of diets with dried oregano (*Origanum vulgare*) leaves had no positive influence on performance or carcass characteristics of lambs. However Bölükbaşi and Erhan (2007) showed that feeding laying hens a diet containing 0.1% and 0.5% thyme improved feed conversion.

Lee et al. (2003) found that some bioactive components of essential oils, especially carvacrol, improved feed conversion ratios in broiler chickens. They proposed that the effect of carvacrol on feed

conversion ratios could be related to increased efficiency of feed utilization.

Low mortality was found by Simonová et al. (2008) during the experimental application of ginseng extracts to rabbits. These findings were similar to our experimental results with the combined supplementation with fennel and thyme, which also appears to decrease digestive disorders while significantly ($P < 0.05$) reducing the mortality rate. A diet enriched with some plant extracts is beneficial for the health rabbits (Simonová et al., 2010). In contrast, Erdelyi et al. (2008) found that combined supplements of essential oils of rosemary (0.15%) and garlic (0.025%) increased mortality. In the study of Eiben et al. (2004), the inclusion of anise and fenugreek seeds at a level of 6 g · $kg^{-1}$ diet increased the pre-weaning mortality in New Zealand white rabbits. However, Gerencsér et al. (2012) observed that supplementing the diet with 3% thyme had no effect on rabbit mortality.

The *Foeniculum vulgare* fruit extracts and oil are rich in *trans*-anethole and other compounds and are effective against *C. albicans,* and other similar organisms (Gulfraz et al., 2008). In the current study, the supplementation of 2.5% thyme and 2.5% fennel and thyme insignificantly decreased caecal counts of when compared with the control group. So the counts of in the caecum of the rabbits were not affected by any of the supplemental treatments, but the supplementation with 2.5% thyme significantly lowered caecal *C. perfringer* populations ($P < 0.05$) as compared with the control and the other group. Bölükbaşi and Erhan (2007) showed that 0.1% and 0.5% of thyme significantly reduced *E. coli* concentrations in the faeces of laying hens.

The essential oils from the thymus species are rich sources of phenolic monoterpenes such as thymol and carvacrol (Karousou et al., 2005; Amiri, 2012). Numerous *in vitro* studies have demonstrated that essential oils, including these molecules, display antimicrobial activity (Celikel and Kavas, 2008; Cosge et al., 2009; Chedia et al., 2013). *C. perfringens* were found to be sensitive to carvacrol, cinnamaldehyde, citral, limonene, thymol, particularly at a higher concentration (500 mg · $l^{-1}$), the same was true for oregano oil, rosemary oil and thyme oil (Ouwehand et al., 2010).

## Conclusions

In this study, dietary supplementation with 2.5% fennel and/or thyme did not affect the growth of rabbits, but significantly reduced their mortality during the fattening period. This reduction could be related to the decrease in bacteria concentrations in the caecum due to the effects of the essential oils contained in these medicinal plants, which must be confirmed by further investigation and research.

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