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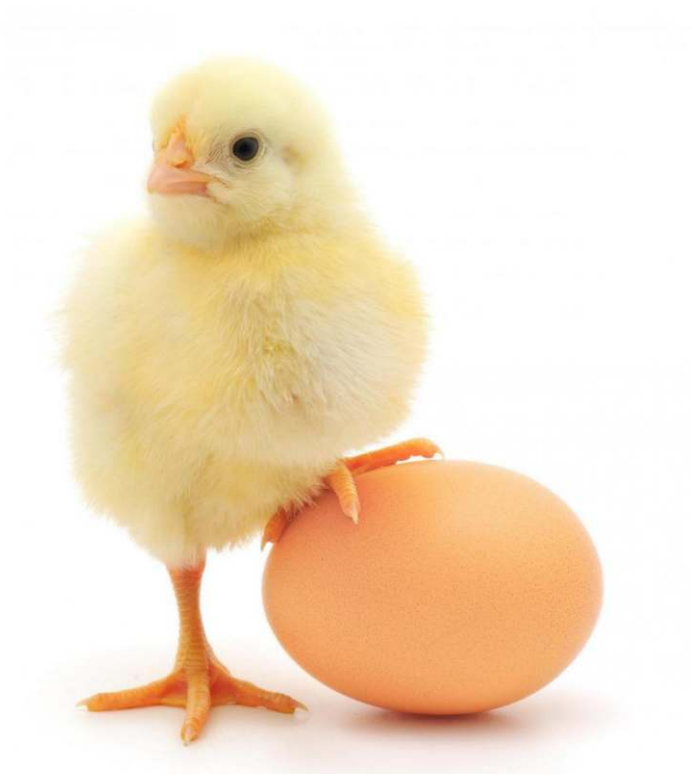

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Effects of Oregano Essential Oil Dietary Supplementation on the Feeding and Drinking Behaviour as Well as the Activity of Broilers

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Abstract: The objective of this study was to evaluate the effect of oregano essential oil dietary supplementation on the feeding and drinking behaviour as well as the activity of broilers. One hundred and twenty female Redbro chicks were randomly assigned to three treatments: Controls, R1 and R2 with 0, 100 and 250 mg oregano essential oil added per Kg of feed, respectively. Their feeding behaviour was recorded from 35-63 days of age. The probabilities of a bird feeding, drinking and moving were significantly decreased by the oregano essential oil supplementation with the higher inclusion level having the greater effect. The age of the chicks and the time of day both had a small but significant negative effect on the visits to the feeder, drinker and the activity of broilers. There was a fairly high positive correlation between the probability of a chicken feeding and drinking and a smaller but still significant correlation between feeding and moving. Oregano essential oil significantly altered feeding behaviour of female broilers with the inclusion level being a significant factor of this change.

Key words: Oregano, feeding behaviour, broiler

INTRODUCTION

Oregano (*Origanum vulgare* L.) essential oil is one of the many plant extracts that are used at present as supplements in animal's diets. It contains mainly carvacrol, thymol and their precursors, γ-terpinene and p-cypene and it possesses intense *in vitro* antimicrobial (Dorman and Deans, 2000), antifungal (Daouk *et al.*, 1995) and antioxidant (Cervato *et al.*, 2000) properties, making it an appropriate candidate as a replacement for antibiotic growth promoters and also a promising food additive in order to prevent meat lipid oxidation. So far, it has demonstrated some encouraging experimental results in broilers (Giannenas *et al.*, 2005) and pigs (Namkung *et al.*, 2004) although, in other reports (Botsoglou *et al.*, 2002; Barreto *et al.*, 2008; Demir *et al.*, 2003; Lewis *et al.*, 2003; Marcincak *et al.*, 2008), it failed to promote growth performance. Furthermore, oregano has been shown to improve meat storage stability after slaughter in poultry (Botsoglou *et al.*, 2003) and rabbits (Botsoglou *et al.*, 2004) and to protect against the negative effects of stress on chicken meat quality characteristics (Young *et al.*, 2003).

Oregano essential oil has a very intense smell. Despite the early suggestions that the sense of smell was either lacking or being very poor in the domestic fowl, there is now compelling behavioural, neurobiological and embryological evidence for a functional olfactory system in chickens (Jones and Roper, 1997). Therefore, when oregano essential oil is added to commercial animal diets, it presents a novel stimulus, which could elicit fear (neophobia) as it has been demonstrated in sheep (Simitzis *et al.*, 2008). This fear could have the potential to harm welfare and performance (Marples and Roper, 1996).

The combination of the potential effects of the dietary supplementation with oregano essential oil, beneficial in terms of production and harmful in terms of welfare, suggests that it is important to investigate its potential consequences on the feeding behavior of farm animals. To our knowledge, there is a very limited amount of literature reports about the effects of dietary supplementation of essential oils on the feeding behaviour of broilers. Therefore, the aim of the present study was to study these effects, if any, on feeding, drinking and activity behaviours of female broilers.

MATERIALS AND METHODS

Animals and diets: One hundred and twenty female medium-growing hybrids (Redbro) were purchased from a local hatchery. On arrival, they were wing-marked and weighed. For the first period (1-28 days of age, growing period) they were housed in batteries with *ad libitum* access to commercial starter feed (in crumbles) and water. The lighting program consisted of 24 h of light for the first two days and 23 h of light-1 h of dark for the rest of the days of this period. Ambient temperature was gradually decreased from 32°C on the first day to 25°C on day 21 and was then kept constant.

At day 28 they were moved and randomly assigned to six identical straw-bedded solid floored cells with the same bell feeders and automated bell drinkers. Feed and water intake remained *ad libitum* but the diet was

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changed to a commercial finisher one in crumbles. The feeders were reloaded with feed daily between 08:00 and 09:00 am. Lighting program was also changed to 16 hours of light (06:00 am to 22:00 pm). The birds were kept under these conditions for one week (28-35 days of age) in order to familiarize with the new environment.

At day 35, the chickens were provided with the oregano supplemented diets. Oregano essential oil used in the present study had a clear dark yellow colour and a herbal, spicy, pungent odour characteristic of oregano (Ecopharm Hellas, SA, Kilkis, Greece). Its main components, as determined by gas chromatography (Shimadzu GC-14A, MSQP2000), were (%): carvacrol (83.10), thymol (2.10), γ-terpinene (3.97), p-cymene (3.79) and β-caryophyllene (0.93). The essential oil was uniformly sprayed on the crumbles with maize oil (1%) as carrier. Three groups were created, control (M) and two levels of oregano essential oil (R1 = 100 and R2 = 250 mg per kg feed). The most common inclusion levels reported in broilers range between 100 and 200 mg/kg (Barreto *et al.*, 2008; Giannenas *et al.*, 2005). Therefore, the doses selected represent the standard (R1) and a high dose (R2). Each group consisted of two replicates, with 20 birds per replicate, so each treatment had 40 animals. Recording procedure took place from the 35^{th} until the 63^{rd} day of age. The analyses of the experimental diets are presented in Table 1.

The experiment was approved by the bioethical committee of the Agricultural University of Athens under the guidelines of "Council Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes".

Recording procedure: The behaviour of the birds was recorded from 06:00 to 22:00 h daily, using three Panasonic PV120 video cameras fitted with a 3.6 mm lens. Each camera was placed in a fixed position in order to record the feeding behaviour patterns in two adjacent cells (1 camera/2 cells). The recorded data were stored in a digital video recorder equipped with a hard disk (TX168, Telexper Inc, USA). After the end of the experiment the following behaviour components were measured: a) The number of birds feeding and drinking per cell, in photos taken every quarter of hour, defined as any bird standing over a feeder or a drinker with its head towards the trough and b) the activity of the birds per cell, defined as the number of birds moving in two adjacent photos with an 1 sec interval. The photos for activity measurements were taken once every hour.

Statistical analysis: Behavioural data were analyzed using random effects logistic regression. We assumed that $M_i \sim \text{Bin}(n, P_i)$, where M_i is the number of the birds feeding, drinking or moving, n the total number of birds per cell, P_i the probability of a random bird feeding, drinking or moving and Bin denotes the binomial distribution. The basic model was as follows:

$$\text{logit}(P_{ijklm}) = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + \zeta_m + \varepsilon_{ijklm}$$

Where logit (P) is the log (p/ [1-p]) transformation, P_{ijklm} is the probability of a chicken feeding, drinking or moving, μ the overall mean, α_i the effect of the cell, β_j the effect of age, γ_k the effect of the time of the day, δ_l the effect of the treatment, ζ_m denotes the random effect for each cell and ε_{ijkl} the residual error. We assumed different random effects for drinking, feeding and moving. In addition, we allowed for the within treatment random effects to be correlated in order to capture some of the dependence in the chickens' behaviour. In addition, we tested whether mean weight per cell was appropriate for inclusion in our model but it appears, based on the Deviance Information Criterion (DIC, Spiegelhalter *et al.*, 2002), that the model that includes weight was inappropriate in the sense that the corresponding DIC was much higher. Statistical analyses were performed using the WINBUGS 1.4.3 software (Bayesian inference Using Gibbs Sampling, Lunn *et al.*, 2000).

RESULTS

Behavioural observations: Table 2 summarizes the effect of the treatment on the feeding behaviour of the broilers. The probability of a bird feeding, drinking and moving was significantly decreased after the dietary oregano essential oil supplementation, with the higher inclusion level (group R2) demonstrating the higher decrease.

Table 1: Analysis of experimental diets

Analysis (%)	Starter	Finisher
Crude protein	22.00	18.00
Fibers	3.25	3.35
Moisture	12.27	13.12
Ash	6.17	5.60
Fat	5.68	3.32
Phosphorus avail.	0.29	0.31
Lysine	1.37	1.00
Methionine	0.57	0.44
Meth + Cyst	0.95	0.77
ME (Mj/kg)	12.31	11.69

Table 2: Mean probabilities (±SE) of a chicken feeding (P_f), drinking (P_W) or moving (P_m) of broilers consumed oregano essential oil supplemented diets (Control = 0, R1 = 100, R2 = 250 mg per kg feed)

	Control	R1	R2
P_f	0.27^a±0.06	0.11^b±0.01	0.04^c±0.01
	(0.17,0.40)	(0.09,0.13)	(0.02,0.07)
P_W	0.92^a±0.02	0.04^b±0.01	0.00014^c±0.00005
	(0.87,0.95)	(0.03,0.05)	(0.00007,0.00025)
P_m	0.42^a±0.13	0.08^b±0.03	0.011^c±0.009
	(0.20,0.68)	(0.04,0.13)	(0.002,0.04)

^{a,b,c}Means within a row with different superscripts are significantly different (p<0.05). ^{d}Numbers in brackets represent 95% confidence intervals

Table 3: Mean model coefficients estimates (±SE) of the probability of a broiler eating (P_f), drinking (P_w) or moving (P_m)

	μ	Cell	Age	Time	Treatment
P_f	-0.4±0.3	0.003±0.023	-0.026±0.001	-0.035±0.003	-0.47±0.22
	(-0.8,0.3)	(-0.04,0.03)	(-0.030,-0.023)	(-0.041,-0.030)	(-0.89,-0.09)
P_w	-3.0±0.3	0.45±0.07	-0.008±0.002	-0.019±0.003	-4.9±0.6
	(-3.4,-2.3)	(0.23,0.52)	(-0.011,-0.004)	(-0.025,-0.012)	(-5.5,-3.1)
P_m	-1.5±0.4	0.06±0.08	-0.039±0.003	-0.046±0.006	-1.8±0.8
	(-2.1,-0.5)	(-0.08,0.18)	(-0.045,-0.032)	(-0.058,-0.034)	(-2.9,-0.3)

[a]Numbers in brackets represent 95% confidence intervals

The model coefficients estimates for the probability of a random chicken feeding, drinking or moving are presented in Table 3. Along with the effect of the treatment, both age and time of the day had a minor negative significant effect on the probabilities, shown by the negative coefficient of the specific component. Accordingly, from the first day of the supplementation till the last, the birds decreased their visits to the feeders and drinkers and their activity. Moreover, all the behavioural parameters mentioned above were gradually decreasing from the beginning to the end of the day.

Table 4 presents the correlations between the probabilities of finding a random bird feeding (P_f), drinking (P_w) and moving (P_m). P_f was significantly and quite highly correlated to P_w and much less but still significantly with P_m (0.60 and 0.19, respectively). In contrast, the probability of a chicken visiting the drinker did not correlate significantly with the probability of a random chicken moving.

Table 4: Correlation coefficients of the probabilities of a chicken feeding (P_f), drinking (P_w) or moving (P_m)

	P_f	P_w	P_m
P_f	1	0.60 (0.50, 0.69)	0.19 (0.07, 0.31)
P_w		1	0.09 (-0.13, 0.30)
P_m			1

[a]Numbers in brackets represent 95% confidence intervals

DISCUSSION

Dietary oregano essential oil supplementation altered the feeding behaviour of the broilers. The chicks from the oregano supplemented groups visited less often the feeders during the whole experiment. Even more, a scaling effect was observed, depending on the inclusion level of the essential oil. If these observations were to be attributed to neophobia, then the average visits of the oregano groups to the feeder should have been raised some days after the supplementation, approaching those of the control group. It has been shown that the supplementation of sheep feed with essential oils caused a significant reduction on feed intake and duration of meals as well as an increase in number of meals but these effects were eliminated a few days later (Simitzis *et al.*, 2005), which is a typical observation in the case of neophobia. In general, farm animals tend to sample cautiously a feed with novel substances and eat it selectively in order to minimize consumption of potentially harmful compounds. This was not the case in our experiment. On the contrary, oregano essential oil's smell and/or taste were not so much appreciated by the birds, because if oregano essential oil dietary supplementation was desirable, the average visits should have risen. Probably, an essential oil with a more desirable smell would have denoted such an effect as it has been reported for sheep and orange oil (Simitzis *et al.*, 2005). As it has been demonstrated in the early work of Kare *et al.* (1957), who tested the acceptance of water containing various flavors, some flavors (strawberry, alfalfa, nutmeg, honey, molasses, mushroom and wild cherry) were rejected outright, while birds would drink certain other flavors (butter pecan, butterscotch, raisin, coconut, grenadine, oil of patchouli and colocynth pulp) sparingly at first, but gradually accept the flavor.

Oregano essential oil dietary supplementation decreased also the visiting rate to the drinkers and this phenomenon was enhanced at the higher inclusion level. It was rather expected though, since there is a direct relationship between feeding and drinking in birds and the two parameters i.e. probability of a chicken feeding and drinking were highly and significantly correlated. If the decreased visiting to the drinkers resulted in less water intake cannot be addressed, since water intake was not measured in this experiment. Nevertheless, Lee *et al.* (2003) found that after supplementing the feed with cinnamaldehyde, water intake was suppressed. As a result, the excreta dry matter was higher, which can be very useful in broiler production in terms of dry litter. Unfortunately, since there are not any other reports in the literature concerning the effect of dietary supplementation with essential oils on feeding behaviour, safe conclusions cannot be drawn and further research is necessary.

Age and time of day were found to express a small but significant negative effect on the probabilities of a chicken feeding, drinking and moving. As the birds grow and get heavier, they spend more time lying or sitting and visited the feeders and drinkers less often (Savory and Lariviere, 2000; Weeks *et al.*, 2000). Moreover, Bokkers and Koene (2003) along with Kristensen *et al.* (2007) also observed that age reduced walking and feeding behaviours in broilers. As far as the time of day effect is concerned, the higher intensity of feeding, drinking and moving in the morning hours probably is firstly due to the absence of feeding during the night and secondly due to the refill of the feeders which occurred daily in the morning hours and acted as stimulation for the birds. Nielsen *et al.* (2003) found that delivering meals to feed restricted broilers increased both feeding and moving behaviours.

Conclusion: The supplementation of broilers' feed with oregano essential oil had significant negative effects on the feeding and drinking behaviour as well as the activity of broilers and also a direct relevance to the inclusion level was identified. On the other hand, the results in the literature about the effects of oregano essential oil dietary supplementation on the growth performance and the meat quality have been promising. Since the feeding behavior and the productive characteristics are close related, further research is necessary for the identification of the inclusion levels of oregano essential oil that promote growth and meat quality without suppressing feeding, drinking or moving behaviors.

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