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Effects of lavender essential oil nebulisation on welfare and nasal microbiota of growing-fattening pigs

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ABSTRACT

Three experimental groups of 36 pigs each (average weight 79 kg) were kept for 127 days in the following experimental conditions: standard farming (C = control), lavender oil nebulisation for 10 min once a day (L1) or lavender oil nebulisation twice a day (L2). No significant differences were observed across groups in growth parameters and chronic stress levels (hair cortisol, dehydroepiandrosterone and their ratio). Behavioural analysis showed similar activity levels across groups, but significant differences for some behaviours: L1 explored the pen floor less than C ($p < .05$); stood inactive more than L2 ($p < .05$) and spent the largest proportion of time eating ($p < .01$). Pigs from group L2 were more frequently observed in a sitting inactive position than C ($p < .05$) and showed a lower interaction with pen-mates and a greater interaction with objects than L1 and C ($p < .05$ and $p < .01$, respectively). A positive effect due to a moderate reduction in aimless exploration of the barren floor could be identified in L1 compared with C ($p < .05$), but the difference did not reach statistical significance in L2. Regarding nasal microbiome, beta diversity was not affected by treatment, while L2 pigs showed tendentially increased alpha diversity compared to C (Chao1 and Shannon indexes, $p = .05$), likely due to changes in rare taxa. Overall, these results suggest that a single or double daily administration may be insufficient to exert a robust positive effect on growth parameters, behavioural traits, stress levels or antimicrobial effect on the pig nasal microbiota, therefore different protocols and/or routes of administration should be assessed.

HIGHLIGHTS

- Pigs exposed to lavender essential oil inhalation once or twice daily did not show variations in growth parameters or chronic stress levels.
- Behaviour and nasal microbiome showed some positive trends but the administration protocol was likely insufficient to determine consistent effects.
- Administration protocols and routes should be optimised to obtain benefits from the anti-anxiety and antimicrobial properties of lavender oil.

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Introduction

Lavender (*Lavandula angustifolia* Miller) essential oil (LEO) is a phytoextract traditionally used in aromatherapy for its calming and anxiolytic effects. As reviewed by Cavanagh and Wilkinson (2002), aromatherapy may act at both the psychological level (effect of the odour) and the physiological level (effects of the inhaled volatile compounds *via* the limbic system, particularly the amygdala and hippocampus). Although the exact

cellular mechanism of action is still unknown, some studies found that in laboratory animals (gerbils and rats) LEO inhalation resulted in anxiolytic effects that were in some cases similar to benzodiazepines (Bradley et al. 2007; Shaw et al. 2011).

Notwithstanding the importance of providing adequate care, management and living conditions to farm animals, the inhalatory administration on LEO could have the potential to help pigs cope with stressful conditions such as group mixing, overcrowding and

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barren environments, having the potential to reduce stress and anxiety in pigs, increase calmness level and overall promoting better animal welfare and performance. Moreover, phytoextracts and essential oils, including LEO, are known to influence bacterial growth. Prolonged exposure to LEO inhalation can affect the nasal microbiota, which plays a vital role in respiratory health by activating immune responses and protecting against pathogens through mechanisms such as colonisation resistance (Elmi et al. 2024).

There is a limited body of literature on the inhalatory administration of LEO to pigs. Most of them refer to the acutely stressing period around transportation, and did not consistently identify positive effects of LEO inhalation on animal behaviour. Bradshaw et al. (1998) provided lavender straw as bedding on the truck, and found increased activity levels during the journey but reduced motion sickness symptoms compared to wheat straw bedding. Crone et al. (2021) attached lavender-infused sachets to the sides of the trailer and found increased standing and mounting behaviours (probably due to pigs trying to reach the enrichment objects) but without an increase in lesions on the body. Direksin et al. (2017) reported that LEO inhalation for 4 h after transportation did not prevent aggressive behaviour, but may have allowed an earlier settlement of hierarchy (i.e. earlier adaptation to the new conditions) compared to the unexposed group.

To the best of our knowledge, only two studies are available exposing pigs to LEO for prolonged periods. In the first study, pigs during the growing phase (40 to 80 kg BW) were exposed to LEO for three 10-min inhalation sessions per day. At the end of the period, no differences were detected in growth parameters, behaviour or chronic stress indicators (steroids from hair) compared to the control group, however a positive effect was observed: pigs exposed to LEO showed fewer lesions on the body (shoulder and thigh region, and total lesions) and reduced severity of tail lesions, possibly indicating reduced aggression and tail biting levels (Elmi et al. 2024). However, nasal microbiome diversity was also reduced, warranting further studies on the microbial modulatory capability of this oil before exploiting it on a larger scale (Elmi et al. 2024).

Another recent study (Nannoni et al. 2023) carried out on finishing heavy pigs (79–160 kg BW) assessed the effects of LEO administered either zero (control), once (L1) or twice (L2) a day (10-min nebulisation sessions) on pig welfare and meat quality. No effects were found on blood stress indicators at slaughter, carcass or meat quality. No lavender residues were found in fat or lean samples, highlighting the

versatility of this administration method for meat-producing animals. In terms of animal welfare, results were inconsistent since at the end of the trial group L1 showed the lowest tail damage, while unexpectedly group L2 had more lesions on the body (in particular on the thighs), leading to a worse lesion score classification. As a possible explanation, the noise and vibration from the nebulisation machine may have disrupted the animals' rest resulting in increased aggressive behaviours in L2 despite the double LEO administration (Nannoni et al. 2023).

This work builds on the previous two experiences on the inhalatory administration of LEO to fattening-finishing pigs on-farm for the entire production phase, aiming to assess more in-depth the effects of LEO inhalatory exposure either once or twice a day on (1) growth parameters; (2) animal behaviour, (3) chronic stress levels assessed from hair and (4) nasal microbiota.

Material and methods

The trial was carried out at the Department of Veterinary Medical Sciences (DIMEVET), University of Bologna, Italy. The experimental protocol was authorised by the Ethical Committee of the University of Bologna as an observational study (no invasive procedure was carried out *in vivo*) with protocol number 3610, date of approval 10 January 2023. Animals were raised in compliance with the EU legislation on the protection of pigs (EC 2008).

Pigs, housing and feeding

The trial included 108 barrows (Goland × Large White crossbreeds) with undocked tails. Ear tags were applied upon arrival to ensure individual identification. Pigs were allotted to three homogeneous experimental groups based on body weight (BW). At the beginning of the trial animals weighed on average 79 kg and were 154 ± 2 days old. Housing consisted of collective pens on a partially slatted floor, each equipped with a nipple drinker, a stainless-steel collective trough and an environmental enrichment tool (soft wooden logs attached to a metal chain). Groups were stable throughout the experimental period. Pens were in temperature- and humidity-controlled rooms (set at 65% RH and 23 °C). Artificial lighting was provided by neon tubes for 12 h a day (0700–1900).

Two commercial feed formulations were used to meet the animals' nutritional requirements. The first formulation (3195 kcal DE/kg DM, CP 14.50% DM) was used up to 110 kg BW and the second (3210 kcal

DE/kg DM, CP 14.20% DM) was used from 110 kg BW to the end of the trial. Meal was rationed at 9.5% of the metabolic BW ($BW^{0.75}$) and feed was provided twice a day (at 8:00 h and 15:00 h) as a liquid obtained by mixing water and meal in a 3:1 ratio. The daily rations were increased every two weeks according to periodical weighing and expected growth rate, up to a maximum of 3.4 kg of meal/head/day.

The farming conditions remained the same until pigs reached an average BW compatible with Parma ham production rules, i.e. approximately 170 kg (Consorzio del Prosciutto di Parma 2023). The trial lasted 127 days.

Experimental design

The three experimental groups included 36 animals each (6 replications of 6 pigs per group). Each group was kept in a different room of the experimental barn to avoid unintended lavender exposure that could act as a confounding factor. While the experimental facility design and the equipment available to carry out the trial (two nebulisation machines) did not make possible to have group replications in different rooms, we made sure conditions and pen design across the three rooms were the same for the three experimental groups and across the trial duration to avoid confounding effects of the room as much as possible. The experimental groups are described below.

- Control (C): no treatment was applied;
- Once-a-day lavender (L1): at 7:00 am, a solution containing 1% lavender (*L. angustifolia* Miller) essential oil (LEO) was vaporised in the room for 10 min;
- Twice-a-day lavender (L2): the 10-min LEO vaporisation took place at 7:00 am and 12:00 pm.

The *L. angustifolia* essential oil (LEO) used for the trial was provided by APA-CT (Forlì, FC, Italy); its chemo-characterization was previously reported (Elmi et al. 2024). and the essential oil was diluted, at the final concentration of 1%, into a water-based mixture containing polyoxyethylene sorbitan monooleate and propylene glycol.

The length of the vaporisation session was determined according to the machine manufacturer's guidelines, considering the room sizes and results from preliminary nebulisation tests, to ensure that all animals received equal exposure to the treatments. The volume of solution consumed for each

vaporisation session was approximately 200 mL. Both the solution and the vaporiser were specifically prototyped for the trial. To avoid possible confounding effects due to pigs anticipating what would happen close to vaporisation times, the distribution of vaporisation sessions throughout the day was planned with the intent to avoid possible associations between LEO vaporisation and feeding time or other events in the daily routine (inspections, room cleaning, etc.). Animals were divided into the different groups a week before the start of the trial, which lasted 127 days.

Growth traits

Individual BW was recorded at the beginning (day 1) and at the end (day 127) of the trial and Average Daily Gain (ADG) was calculated. Feed intake of each pen was recorded to calculate the Feed Conversion Ratio (FCR).

Behavioural recordings

On days 7, 77 and 126 (beginning, middle and one day before the end of the trial), four pens per experimental group were videotaped from 6:30 am to 6:30 pm using a digital closed-circuit system (DSE, Turin, Italy) with cameras mounted above the pens. To allow for individual recognition, a coloured spot was painted on the back of each pig using markers for pigs (Raidex GmbH, Dettingen an der Erms, Germany). All videos were stored on a hard drive. A single trained observer analysed the videos (scan sampling every 10 min) according to the ethogram described by Nannoni et al. (2019) including the following behaviours: standing inactive, sitting inactive, sternal recumbency, lateral recumbency, walking, eating, drinking, rooting/exploring the floor, interaction with the enrichment device, interaction with other pen structures. When they occurred in the observation interval, social interactions (sum of positive, neutral and aggressive contacts) and tail biting were also noted. However, the correct description and interpretation of these behaviours would require a different methodological approach (continuous observation of the whole group or focal animals) which falls outside the aim of the present study. Results are reported as percentage of observed time for each behaviour.

Hair cortisol and dehydroepiandrosterone (DHEA)

For each experimental group, a sub-sample of 24 pigs was randomly selected. Animals were shaved off

manually at the beginning of the trial (day 0). The re-grown bristles were sampled at the end of the trial (day 127) to quantify of cortisol and DHEA as previously reported (Bacci et al. 2014; Elmi et al. 2020; Graziosi et al. 2024). Briefly, hair samples (250 mg) were washed with water and isopropanol in order to remove any organic residue from the surface. Once fully dried, samples were finely pulverised (120 mg) and extracted overnight in 4 mL of methanol. The supernatant was collected and evaporated. The dry extracts were reconstituted in assay buffer (phosphate buffered saline, 0.1% BSA, pH 7.4) for measurement of cortisol (6 mg hair equivalent) and DHEA (Dehydroepiandrosterone) (4 mg hair equivalent) by radioimmunoassay as previously described (Elmi et al. 2024).

Nasal microbiota

Sterile swabs (APTACA Spa, Italy) were used to collect samples for nasal microbiota analyses. On day 127, 8 animals per experimental group were restrained and swabs were inserted for approximately 5 cm into each nostril and rotated for 5 s. In case of accidental touch with the snout or other surfaces, swabs were discharged and procedure was repeated. Swabs were store at -80°C until analyses. For the microbiota analysis, bacterial DNA extraction from nasal swabs was carried out using FastDNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, Ca, USA) adding an initial step where dry swabs were soaked in a Sodium Phosphate Buffer solution and vortexed for 5' in order to resuspend microbial cells, the following steps were carried out according to the manufacturer's instructions. DNA concentration and purity (absorbance ratio 260/280 and 260/230) of the isolated DNA were checked by spectrophotometry on the NanoDrop (Fisher Scientific, 13 Schwerte, Germany). The V3–V4 region of the 16S rRNA gene (~460 bp) was amplified, amplicons were produced using the universal primers Pro341F: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTACGGGNBGCASCAG-3' and Pro805R: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTA CNVGGGTATCTAATCC-3' (Takahashi et al. 2014) using the Platinum™ Taq DNA Polymerase High Fidelity (Termo Fisher Scientific, Italy) and sequenced using the Illumina MisSeq platform 300x2bp. The libraries were prepared using the standard protocol for MiSeq Reagent Kit V3 and sequenced on MiSeq platform (Illumina Inc., San Diego, Ca, USA). For the bioinformatics analysis, the DADA2 pipeline was used (Callahan et al. 2016) considering the Silva database (Quast et al. 2013) (version 138.1) as reference for the taxonomic assignment.

Statistical analysis

Statistical analysis and graphical representations were carried out using the software Statistica, release 12 (StatSoft Inc., Tulsa, OK, USA) and GraphPad Prism v.9 (GraphPad Software Inc., San Diego, CA, USA). Descriptive statistics were reported as Mean \pm standard deviation (SD). Normal distribution was assessed using the Shapiro–Wilk test ($p < .05$). One-way ANOVA was applied to continuous data, using the experimental group as the main factor. Pairwise comparisons were then carried out using the Bonferroni test when significant differences were found. The experimental unit was the pen for growth parameters and the individual for behavioural observations and lesions on the body. The significance level was set at $p < .05$ for all tests.

The statistical analysis on Alpha diversity, Beta diversity and taxonomic composition was carried out with R v3.6. Alpha diversity, Chao1, Shannon, and Simpson diversity indices were measured and differences were tested using a linear model including sequencing depth and treatment (C, L1 and L2) as factor.

For the Beta diversity a dissimilarity matrix using Euclidean distances of centred log ratio (clr) transformed data was constructed, results were plotted using a NMDS plot. Differences were tested using a PERMANOVA model with 10,000 permutations, including the effect of treatment (C, L1 and L2).

The differential abundance analysis was performed using Linear discriminant analysis Effect Size (LEfSe)(Segata et al. 2011) implemented in the wrapper function included in the package microbiomeMarker (Yang 2020), aggregating the data at Genus level. Using a LDA cut-off of 3.5 and $P_{\text{adj}} < 0.05$. p Values $< .05$ were considered statistically significant while p values $< .1$ were considered a trend of significance.

Results

Animals showed no health issues that may have altered the trial results and interpretation.

No significant differences were observed among the experimental groups in any of the growth parameters recorded (body weight, average daily gain, feed conversion ratio) (Table 1).

Table 2 shows the time budgets resulting from behavioural observations. The overall level of activity did not differ among the experimental groups, with animals spending in recumbency an almost identical amount of time (approximately 67%, $p = .942$) across the three groups. Significant differences were

observed for some behaviours. Group L1 spent significantly less time exploring the pen floor than group C and carried out significantly more total interactions with pen-mates (sum of neutral, positive and aggressive social interactions and tail biting) compared to L2 ($p < .05$ for both differences). Group L1 spent significantly more time standing inactive ($p < .05$) and carrying out total social interactions ($p < .01$) than group L2. Group L1 also spent a higher proportion of time eating ($p < .01$) compared to both other groups. Group L2 spent more time sitting inactive than C ($p < .05$) and the greatest objects exploration time (both pen fixtures and the enrichment tool) compared to the other groups ($p < .01$).

Table 3 shows the hormonal analyses carried out on bristles sampled at the end of the trial. No statistical difference was observed in cortisol or DHEA concentrations, and their ratio, among the experimental groups.

A total of 26, 627 quality checked reads were assigned to 1435 different ASVs. For the taxonomic composition a total of 22 Phyla were identified: Proteobacteria 50.97 ± 4.94%, Firmicutes 33.88 ± 1.03%, Bacteroidota 9.20 ± 0.96%, Actinobacteriota 4.56 ± 0.56% and Euryarchaeota 0.43 ± 0.16% were the most represented. At family level a total of 117 families were identified:

Moraxellaceae 49.32 ± 6.3%, Clostridiaceae 9.96 ± 1.77%, Streptococcaceae 7.38 ± 3.1%, Weeksellaceae 5.52 ± 1.97% and Lactobacillaceae 4.41 ± 1.3% were the most abundant. At Genus level a total of 238 genera were identified: *Moraxella* 45.31 ± 9.62%, *Clostridium_sensu_stricto_1* 9.76 ± 1.99%, *Streptococcus* 7.35 ± 3.30%, *Lactobacillus* 4.41 ± 1.3% and *Weissella* 3.21 ± 0.45% were the most abundant.

Alpha diversity measures are represented in Figure 1. Chao1 and Shannon tended to be affected by treatment ($p = .06$), with a tendency of a lower diversity in C compared to L2 ($p = .05$).

For the beta diversity, PCoA plots generated using the Euclidean distance matrix between samples, are reported in Figure 2. Bacterial composition was not affected by treatment, however pairwise adonis test suggested a significantly different bacterial composition between L1 and L2 ($R^2 = 0.16$, $p = .048$).

Results for the LEfSe analysis are reported in Figure 3. Subjects from L1 group were characterised by a higher abundance of *Acinetobacter* (LDA_score = 4.36, P.adj = 0.02), *Lysinibacillus* (LDA_score = 3.81, P.adj < 0.01), *Psychrobacter* (LDA_score = 3.76, P.adj = 0.01), *Flavobacterium* (LDA_score = 3.67, P.adj = 0.03) and *Jeotgalibaca* (LDA_score = 3.59, P.adj = 0.01). On the other hand, subjects from L2 were

Table 1. Growth parameters of the three experimental groups (C = control, L1 = lavender nebulisation once a day, L2 = lavender nebulisation twice a day).

Treatment	C	L1	L2	SEM ¹	p-value
Replications, n.	6	6	6	–	–
Initial weight, Kg	79.18	82.63	79.74	1.428	0.941
Final weight, Kg	167.0	171.8	170.4	1.167	0.263
Average Daily Gain (ADG), Kg	0.691	0.702	0.714	0.006	0.313
Feed consumption, Kg/d	2.91	2.91	2.93	0.007	0.976
Feed Conversion Ratio (FCR)	4.21	4.14	4.10	0.040	0.711

¹Standard Error of the Mean.

Table 3. Cortisol and DHEA (dehydroepiandrosterone) and their ratio from hair (sampled at the end of the trial) of the three experimental groups (C = control, L1 = lavender nebulisation once a day, L2 = lavender nebulisation twice a day).

Treatment	C	L1	L2	Pooled SEM ¹	p-value
Samples, n.	24	24	24	–	–
Cortisol, pg/mg	9.28	11.06	10.12	2.455	0.8354
DHEA, pg/mg	27.74	30.12	28.93	3.806	0.4616
Cortisol/DHEA	0.35	0.37	0.37	0.097	0.6629

¹Standard Error of the Mean.

Table 2. Behavioural observations over the diurnal hours (7 AM – 7 PM) of the three experimental groups (C = control, L1 = lavender nebulisation once a day, L2 = lavender nebulisation twice a day).

Treatment	C	L1	L2	Pooled SEM ¹	p-value
Animals, n.	24	24	24	–	–
Standing inactive	4.32 ^{ab}	5.34 ^a	4.06 ^b	0.216	0.0372
Sitting inactive	1.56 ^b	2.45 ^{ab}	2.63 ^a	0.176	0.0301
Lateral recumbency	28.34	32.25	30.04	0.702	0.0730
Sternal recumbency	38.58	35.05	36.665	0.603	0.0566
TOTAL RECUMBENCY ²	66.92	67.30	66.70	0.719	0.9419
Eating	4.19 ^B	4.73 ^A	4.02 ^B	0.747	0.0002
Drinking	0.14	0.17	0.34	0.042	0.1133
Walking	0.89	0.77	0.83	0.083	0.8527
Exploring (sniffing/rooting) the floor	11.92 ^a	8.53 ^b	10.91 ^{ab}	0.477	0.0110
Total interactions ³	4.17 ^a	5.11 ^a	2.52 ^b	0.245	0.00005
Total object exploration ⁴	5.90 ^B	5.59 ^B	7.99 ^A	0.298	0.0015

Data are expressed as a percentage of the observed behaviours. Different superscripts within the same row indicate significant differences (^{a,b} $p < 0.05$; ^{A,B} $p < 0.01$).

¹Standard Error of the Mean.

²Total recumbency = Lateral + Sternal recumbency.

³Total interactions = Social interactions (neutral, positive and aggressive) + Tail biting.

⁴Total object exploration = Exploration of the enrichment + Exploration of pen fixtures.

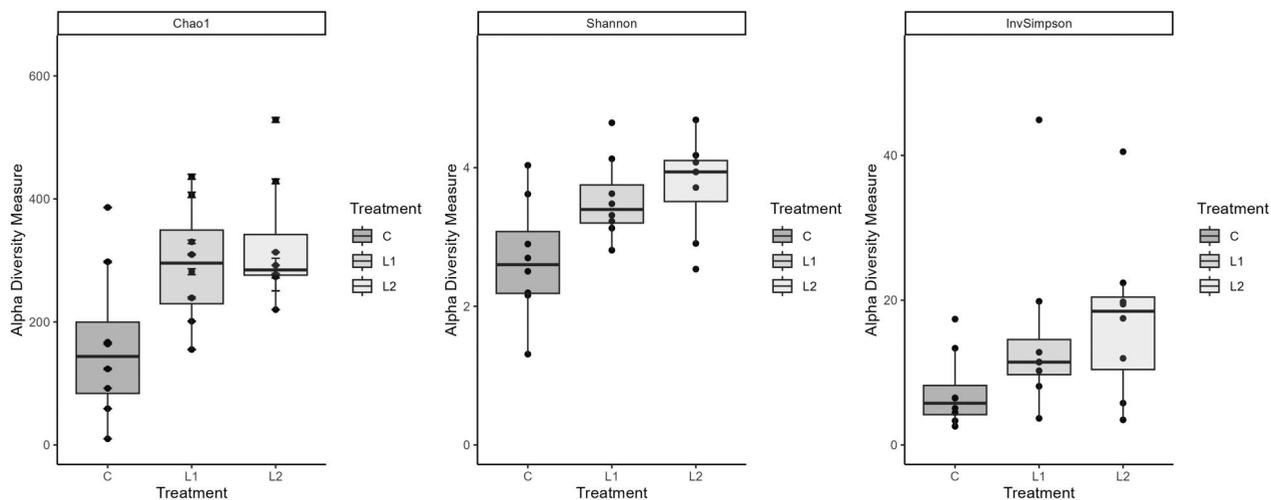


Figure 1. Boxplots showing the alpha diversity measure of the three experimental groups (C = control, L1 = lavender nebulisation once a day, L2 = lavender nebulisation twice a day).

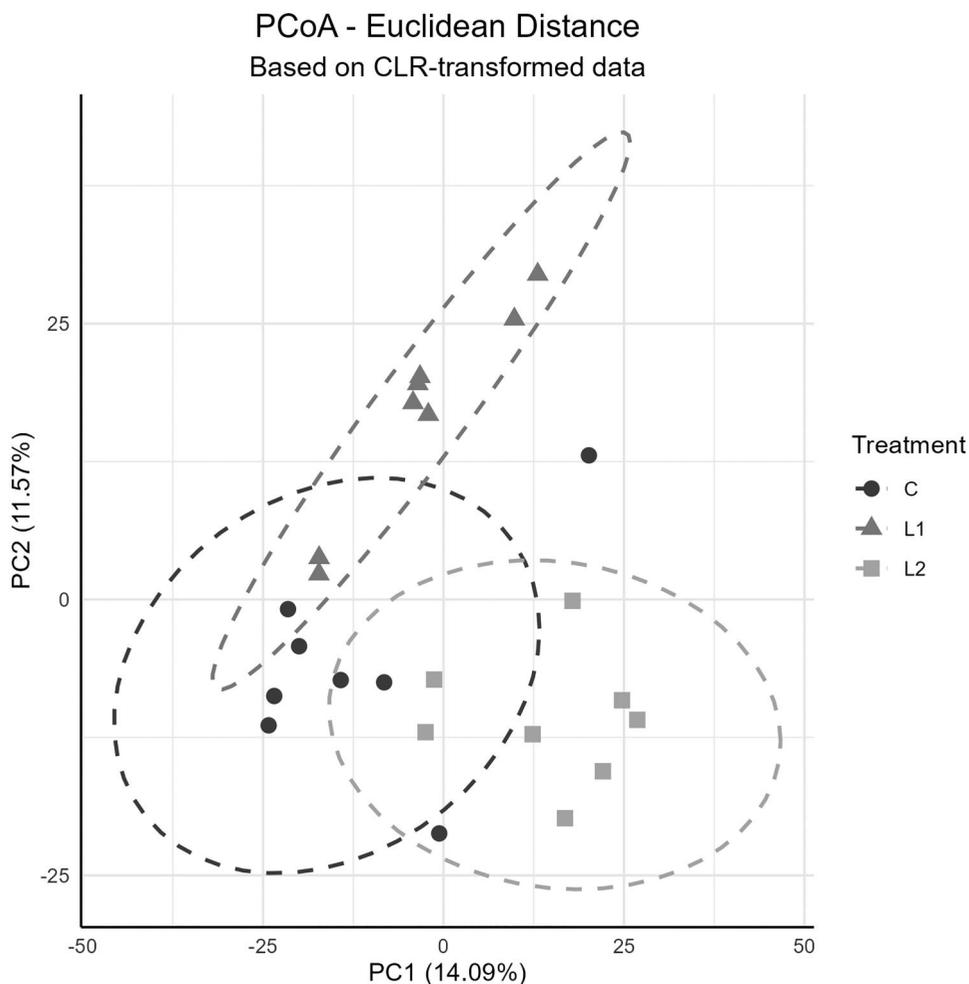


Figure 2. PCoA Plot showing the beta diversity measured with Euclidean distance between 'clr' transformed abundance of the three experimental groups (C=control, L1 = lavender nebulisation once a day, L2 = lavender nebulisation twice a day).

characterised by a higher abundance of *Clostridium_sensu_stricto_1* (LDA_score = 4.69, P.adj = 0.02), *Chryseobacterium* (LDA_score = 4.37, P.adj < 0.01),

Turicibacter (LDA_score = 4.02, P.adj = 0.03), Rikenellaceae_RC9_gut_group (LDA_score = 3.65, P.adj = 0.02), W5053 (LDA_score = 3.58, P.adj = 0.01)

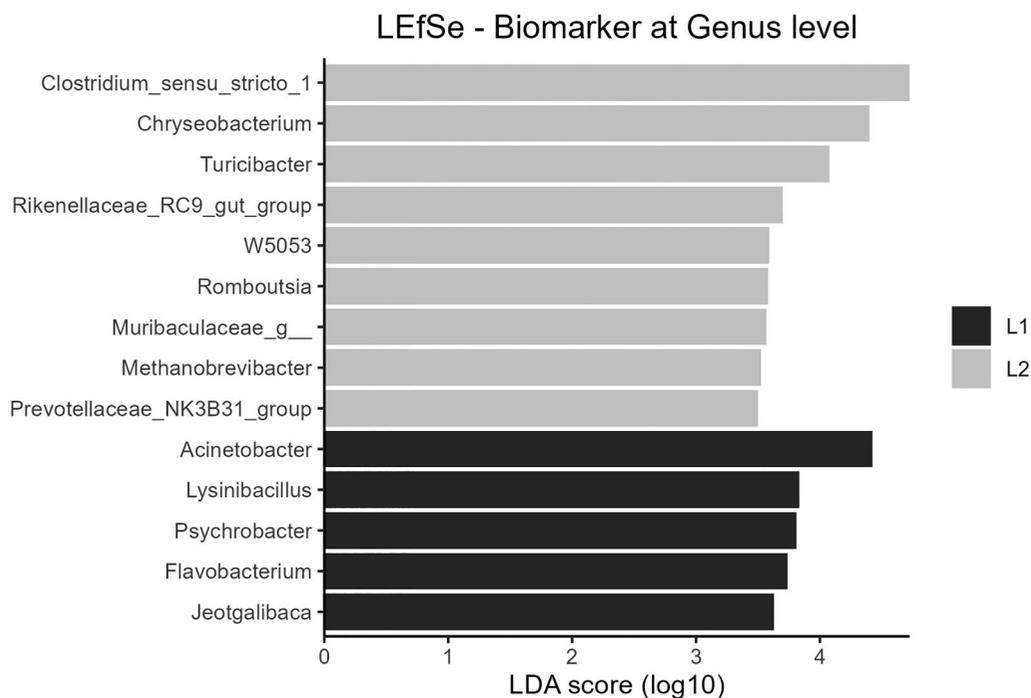


Figure 3. Linear discriminant analysis effect size (LEfSe) plots of the biomarker genera for of the three experimental groups (C = control, L1 = lavender nebulisation once a day, L2 = lavender nebulisation twice a day).

Muribaculaceae (uncultured genera) (LDA_score = 3.53, P.adj = 0.03) and *Romboutsia* (LDA_score = 3.52, P.adj = 0.04).

Discussion

While being less favourable (lower ADG due to the higher BW at slaughter, higher FCR) compared to the average in other European countries (AHDB 2024), the growth parameters recorded in the present study agree with studies carried out on Italian heavy pigs in the same body weight range (Gallo et al. 2014; Nannoni et al. 2019). It should in fact be highlighted that, to comply with the cured ham production rules requiring a minimum age at slaughter of nine months and an average carcass weight between 110.1 and 168.0 kg (Consorzio del Prosciutto di Parma 2023), pigs were fed on a rationed diet.

No difference due to lavender inhalation was observed in terms of growth parameters. These results differ from what had been previously found in other species (broilers), where an improvement in growth parameters was reported after lavender extract administration either in water (improved BW and FCR) (Adaszynska-Skwirzynska and Szczerbinska 2019) or in feed (increased feed intake, BW gain and FCR) (Salarmoini et al. 2019). Inevitably, it is likely the rationed feed regimen had an important role in the lack of difference observed. It should however also be

pointed out that the inhalation administration route used in the present study may have resulted in a less powerful effect (or at least not in a direct effect on the gastrointestinal tract) compared to the oral administration that proved to be effective in poultry. This lack of difference in growth performance agrees with previous studies on LEO aromatherapy in pigs, who did not highlight effects on growth traits in growing pigs (Elmi et al. 2024) or on carcass traits in heavy pigs (Nannoni et al. 2023).

Concerning behavioural observations, the ethogram was in good agreement with previous observations carried out on Italian heavy pigs with the same technique and ethogram (Nannoni et al. 2019). The time animals spent resting (lying down in lateral or sternal recumbency position) was very similar across groups, implying that the overall activity level was not affected by LEO inhalation. As reviewed by de Sousa et al. (2015), in rats LEO inhalation reduced anxiety (e.g. by increasing open arms exploration in the elevated plus maze), but the effect on total motor activity was less investigated. However, studies on rats found that LEO inhalation reduced peripheral movement and defaecation immediately after exposure, while the effects on mobility were not consistent: no effects in one case (Shaw et al. 2011), increased immobility in the other (Shaw et al. 2007). To the best of our knowledge, the only study available reporting time budgets shows no behavioural differences between the control

and the lavender-exposed group in growing pigs receiving LEO nebulisations three times a day (Elmi et al. 2024). However, in the mentioned study the sham group (exposed to the same nebulisation protocol but only using the propylene glycol vehicle, without LEO), was more active (lower lateral recumbency time compared to the lavender-exposed group) and carried out more neutral social interactions compared to both other groups. In the present study, group L1 spent more time eating and less exploring the pen floor less than C, while group L2 showed dog-sitting position more, carried out less interactions with penmates and more object exploration compared to C. Dog-sitting is generally considered as a stereotypical behaviour and an indicator of discomfort or sub-optimal welfare (Cagienard et al. 2005). Overall, these findings do not allow to draw strong conclusions on the behavioural effects of LEO inhalation on diurnal behaviour.

If we consider the main activity that pigs carry out when they are awake, i.e. exploring the floor (8–12% of the diurnal hours), we can observe that pigs in the control group carried out this behaviour more than the other two groups, with a significant difference if compared to L1. This seems to indicate a reduction in aimless, restless activities (exploring a barren, slatted floor) that may be due to the calming-antianxiety effects of lavender. The overexpression of this behaviour (together with sniffing/rooting pen fixtures) is in fact considered to be abnormal and to reflect reduced welfare (Bøe 1993). However, as previously mentioned, this difference in the time budget was observed only between C and L1, while with L2 the difference was merely numerical. Overall, if a slightly positive effect of LEO inhalation on animal behaviour can be detected, this seems to be more the case in L1 (one administration per day) than in L2 (two administrations per day). This observation agrees with previously reported findings (Nannoni et al. 2023) showing that at the end of the trial, group L2 had more lesions on the entire body compared to C, with L1 being intermediate, and tails in L2 were more damaged compared to L1. While the overall resting time remained stable across groups, it is possible that the pigs' activities may have been somehow disrupted by the nebulisation sessions twice a day, determining a slightly increased restlessness and masking the positive effects of the treatment by increasing their activity during and/or immediately after the nebulisation sessions.

On the contrary, in growing pigs (40–80 kg), lavender administration three times a day had led to a reduction in the overall lesion score and a

considerably reduced number and severity of tail lesions compared to control (Elmi et al. 2024). It is therefore likely that the positive behavioural effects of lavender administration on aggressive behaviours and tail biting are more pronounced in younger animals.

Hormonal results on bristles do not indicate any difference across groups, highlighting the absence of positive effects of the treatment on chronic stress levels, but also the absence of a chronically stressful effect caused by lavender administration. This is true both for cortisol from bristles (chosen as a long-term stress indicator, as suggested by Bacci et al. 2014) and DHEA (chosen for its role as an 'anti-stress hormone'). DHEA levels are expected to increase during an acute stress event and decrease during chronic stress (Fels et al. 2019). The hair Cortisol and DHEA concentrations are aligned with previous findings reported in same-age pigs (Bergamin et al. 2019). On the other hand, the Cortisol/DHEA ratio, which better represents the Hypothalamic-pituitary-adrenocortical axis and is considered a welfare indicator in pigs (Trevisan et al. 2017), did not change, unlike what was observed by Elmi et al. (2024) in growing pigs treated with lavender essential oil.

The results of the study on the nasal microbiota demonstrated that administering lavender treatment once or twice daily did not significantly affect the beta diversity of the pig nasal microbiome compared to the control group. This finding contrasts with a previous study where administering lavender three times a day significantly altered the microbial structure. However, the current study revealed a notable increase in alpha diversity in pigs receiving lavender supplementation twice daily compared to controls, differing from earlier findings. Specifically, the Chao1 and Shannon indexes showed significant increases, while the InvSimpson index remained unaffected. This discrepancy highlights that the observed diversity shifts are likely due to changes in rare taxa, as the indexes differ in their sensitivity to rare species. These results suggest that a single or double daily administration may be insufficient to exert a robust antimicrobial effect on the pig nasal microbiota, potentially due to reduced exposure compared to the previous study.

Interestingly, certain bacterial genera were enriched following single or double administrations of lavender. Among these, *Turicibacter*, previously associated with lavender treatment in earlier trials, exhibited a notable increase. Additionally, genera such as *Clostridium sensu stricto-1*, *Acinetobacter*, *Chryseobacterium*, and *Flavobacterium* also showed elevated levels with lavender treatment. These taxa form part of the core

nasal microbiota in pigs and are known for their diverse functional roles.

Flavobacterium and *Clostridium sensu stricto-1* are notable components of the porcine nasal microbiota, demonstrating potential roles in maintaining microbial ecosystem stability. Studies indicate that *Clostridium sensu stricto-1* is negatively associated with virulent strains of *Glaesserella parasuis* (formerly *Haemophilus parasuis*), a pathogen linked to respiratory diseases in swine. Conversely, members of the Flavobacteriaceae are positively associated with non-virulent bacterial strains in the nasal cavity, suggesting their ability to modulate microbial interactions to favour non-pathogenic communities. This dynamic indicates their potential as stabilising factors, reducing the proliferation of virulent strains and potentially mitigating disease risk (Mahmmod et al. 2020). Similarly, *Acinetobacter* is recognised as a dominant genus within the healthy porcine nasal microbiota, contributing to homeostasis and forming an integral part of the core bacterial community in pigs' noses (Yue et al. 2011).

Though less frequently detected in the nasal microbiota, species of *Chryseobacterium*, some species are opportunistic pathogens and are also known to promote inflammation through the stimulation of macrophages (Hou et al. 2022).

Conclusions

The effects obtained with the inhalatory administration of lavender essential oil either once or twice a day to growing-fattening pigs were inconsistent. While some positive outcomes were observed in terms of behavioural observations in group L1 (reduction in time spent exploring the pen floor), those effects were not observed in L2. Similarly, as concerns nasal microbiome, beta diversity was not affected by treatment, while L2 pigs showed tendentially increased alpha diversity. These results, together with the absence of significant variations in growth parameters or hair cortisol and DHEA, suggest that a single or double daily administration may be insufficient to exert a robust positive effect on the observed parameters. While on-field trials may help assess the presence of possible positive effects on larger numbers of animals and replicates, different protocols and/or routes of administration should be tested in order to exploit the beneficial effects of the anti-anxiety, calming and antimicrobial properties of lavender essential oil. Additional aspects to consider in future studies could be a post-nebulisation verification of the LEO concentration achieved in the rooms, and a longitudinal behavioural study to assess whether the

effects of LEO nebulisation on pigs' behaviour change over the fattening cycle.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability and data deposition

Endocrinologic and behavioural raw data supporting the findings of this study are openly available in AMSActa Institutional Research Repository at <https://doi.org/10.6092/unibo/amsacta/8145>. Raw sequences are available at <https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1218946>.

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