

Effects of disinfectant fogging procedure on dust, ammonia concentration, aerobic bacteria and fungal spores in a farrowing-weaning room

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Abstract

Introduction and Objective. In the last decades, large-scale swine production has led to intensive rearing systems in which air quality can be easily degraded by aerial contaminants that can pose a health risk to the pigs and farm workers. This study evaluated the effects of fogging disinfectant procedure on productive performance, ammonia and dust concentration, aerobic bacteria and fungal spores spreading in the farrowing-weaning room.

Materials and Method. This trial was conducted in 2 identical farrowing-weaning rooms of a piggery. In both rooms, 30 pregnant sows were lodged in individual cages. At 75 days of age, the piglets were moved to the fattening room. In the treated room, with the birth of the first suckling-pig, the fogging disinfection with diluted Virkon S was applied once a day in the experimental room per 15 minutes at 11:00. The fogging disinfectant treatment was switched between rooms at the end of the first trial period. Temperature, relative humidity, dust (TSP-RF fractions and number of particles), ammonia concentration and aerial contaminants (enterococci, Micrococcaee and fungal spores) were monitored in both rooms.

Results. Ammonia concentration reduction induced by fogging disinfection was estimated 18%, total suspended particles and the respirable fraction were significantly lower in the experimental room.

Fungal spores resulted in a significant reduction by the fogging procedure, together with dust respirable fraction and fine particulate matter abatement.

Conclusions. The fogging disinfection procedure improved air quality in the piggery, thereby enhancing workers and animals health.

Key words

disinfectant fogging, piggery, dust, ammonia, aerobic bacteria, fungal spores

INTRODUCTION

In the last decades, large-scale swine production and animal genetic selection have increased the animals' production potential; however, at the same time, they have reduced the rusticity [1] of the animals lodged in buildings where air quality can be easily degraded by aerial contaminants, thereby posing a health risk to the pigs and farm workers [2].

Aerial contaminants generated in the pig buildings are classified into particulates, gases, and airborne microorganisms [3]. Dust, or particulate matter, represents an important aerial contaminant in piggeries, since it can combine with inorganic compounds, gases, bacteria and viable endotoxins, which fastened on to the surface of dust particles, can become potentially hazardous agents [4]; this risk may affect piggery workers who may show signs of immune system activation, as well as overt respiratory disease [5]. From an occupational health point of view, dust is classified by size into 3 primary categories: 1) Respirable Dust, 2) Inhalable 3) Dust and Total Dust.

- 1) Respirable Dust refers to those dust particles that are small enough to penetrate the nose and upper respiratory system and deep into the lungs.
- 2) Inhalable Dust is intended as that size fraction of dust which enters the body, but is trapped in the nose, throat, and upper respiratory tract; the median aerodynamic diameter of this dust is about 10 µm.
- 3) Total Dust or Total Suspended Particles includes all airborne particles, regardless of their size or composition.

Ammonia, mainly generated by the enzymatic decomposition of urea from urine, at high concentration can affect pigs' health and performance and is an important cofactor in the genesis of atrophic rhinitis and enzootic bronchopneumonia [6, 7]. In particular, in piggery workers, exposure to dusts and ammonia has shown to be associated with chronic inflammation of the airways and respiratory function impairment [8].

Airborne microorganisms in pig buildings may exhibit a significant impact on human and animal health, more than 80% of the airborne microorganisms found in cattle, pig, and poultry housing are staphylococci and streptococci, fungi, and moulds, yeasts can be more than 1% and coliform bacteria about 0.5% of the total aerobic count [4].

Bacteria like *Enterococci* (*E. faecalis* and *E. faecium*) and fungi, both present in the swine environment, respectively

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can account for the majority of infections (79%), and release allergens, toxins and odours into the indoor air [9].

In animal houses, a widespread environmental disinfection procedure is represented by a rooms fogging system, after cleaning them with water. Recent research conducted in the food industry have shown that fogging disinfectant is effective in reducing the number of organisms on upward-facing surfaces but, in general, it is not effective on vertical or downward-facing surfaces [11]. Fogging also reduces the number of viable airborne organisms, although the reason for this decrease is not understood.

The presented study evaluated the effects of fogging disinfectant procedure on productive performance, ammonia and dust concentration, aerobic bacteria and fungal spores spreading in a farrowing-weaning room.

MATERIALS AND METHODS

Description of the monitored building and animals. The trial was carried out in a piggery for Parma ham production (pigs of around 150 kg at slaughtering age) in Northern Italy. The study was conducted in parallel in 2 rooms, the first time during the autumn and then a replicate of the trial was performed in winter, switching fogging between rooms, to avoid undesired effects depending on room type.

Two identical adjacent farrowing-weaning rooms consisting of 30 cages each were used in the experiment. The rooms had a fully-slatted floor with a slurry pit beneath. Manure was removed at the end of the production cycle.

Fifteen primiparous and 15 pluriparous sows entered in the farrowing room 3–4 days before delivering piglets; 20% of the piglets, previously marked by tattoos, were individually weighed at birth, at 15 days of age, at 30 days of age and at 75 days of age.

Weaning took place when the sows were removed at day 28. After the weaning phase, at 75 days of age, the piglets were moved to the fattening room.

Animals from both rooms were given the same diet (Tab. 1, 2).

Table 1. Formulation of sows diet

DIET	% of total diet
Corn meal	51
Soybean meal	17
Wheat bran	9
Wheat flour milling	7
Rice flour milling	5
Molasses	2
Cereals byproducts	4
Animal fat	1
Vitamin mineral premix	1.5
Calcium carbonate	1.5
Bicalcium phosphate	1
Total	100

Bio-security routine farm procedures. Before the beginning of the trial, both rooms were washed with running water and treated with a bacteriocidic and virucidic product (Antec HD3, Antec International Limited, Sudbury, Suffolk, UK)

Table 2. Formulation of piglets diet

DIET	% of total diet		
	Prestarter	Starter	Weaners
Barley	36	17.5	10
Wheat	15.5	43	4.5
Corn	5		26
Whey powder	4		
Soybean meal	7.8	6.2	12
Toasted soybeans	5.5		
Beat pulp			6
Soybean meal	7.8	6.2	12
Toasted soybeans	5.5		
Beat pulp			6
Extracted sunflower seed			7.8
Extracted rape seed			9.7
Rice flour milling			5
Wheat bran	2.5	7.8	20
Potato protein		3.5	
Molasses			2.5
Sunflower meal			3.2
Alfalfa			6.5
Linseed	1.7		
Corn treated		9.6	
Fish meal	0.5		
Fat (animal)	3.83	5.11	
Soy oil	0.5		
Limestone	0.91	0.92	0.8
Monocalcium phosphate	0.85		1.5
Salt	0.46	0.53	0.5
Vitamin and mineral premix	1.85	1.44	1.5
Total	100	100	100

diluted 1:200. The pit and the channels for the excretions were also emptied and washed according routine biosecurity procedure (all in /all out) adopted in the farm. Before entering the rooms, the sows were washed with running water, then disinfected with a low pressure water pump through nebulisation of VIRKON S (Antec INT., diluted 1:200).

Bio-security Treatment. In the experimental room, starting with the birth of the first suckling-pig, the environmental treatment (spraying of Virkon S, diluted 1:200 in the experimental room through fogging system) was applied once a day per 15 minutes, at 11:00. The overall experimental period lasted 152 days.

Environmental parameters. Measurements of temperature (T, °C) and relative humidity (RH, %) were taken for both rooms, using a specific psychrometric probe (BSV102, Lsi Instruments, Milan, Italy) connected to a data-recorder (Babuc M, Lsi Instruments, Milan, Italy), set for hourly readings. Probes were positioned in the centre of the rooms at a height of 1.70 m. The probe had an accuracy of 0.3 °C for temperature, and an accuracy of 3% for RH.

The ventilation rate of the rooms, temperature controlled, was measured using a portable fan-anemometer, BSV202 (LSI Instruments, Milan, Italy), with a measuring accuracy of 5%. To create the curve of the ventilation load, the speed of the

single fans were measured, setting their absorbance manually, 3 readings were taken at each speed and the average calculated.

Ammonia measurements. The ammonia concentration inside the 2 rooms was measured by passive diffusion sampling (Draeger tubes system, Draeger Safety Inc., Pittsburgh, USA), each measurement lasted 24 hours and the measured values were the expression of mean ammonia concentration during the 24 hours of exposure.

The tubes, were placed at a height of approximately 1.7 m from the slatted floor at 3 measurements points in each room, near the entrance door (1), in the middle of the room (2), at the end of the room (3) (Fig. 1).

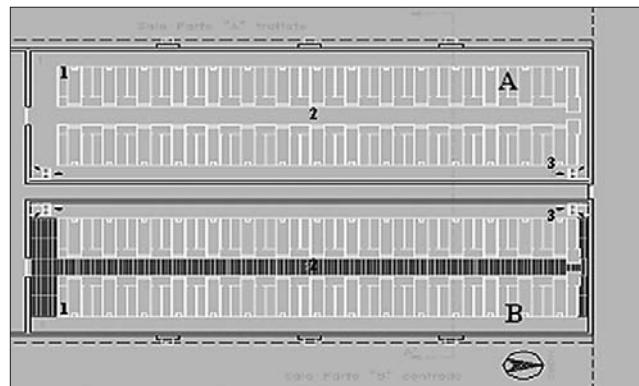


Figure 1. Top view of the two porcine farrowing-weaning rooms and sampling points in the study.

2 – sampling points of environmental parameters (Temperature and Relative humidity) and dust collected by Hyac Royco (estimation of dust particle size fraction)

1,2,3 – sampling points of dust, ammonia and airborne microorganisms.

Measurements began with the sows delivering (day 0) and then a further 4 times, on days 15, 30, 60 and 75, until the piglets were moved to the fattening room.

Dust measurements. Dust measurements were performed using 2 different apparatuses. The first was a 2-stage Lippman cyclone connected to a pump with a counter-positioned piston, set at a constant flow rate (10 l min^{-1}) with an air flow monitoring-counting system. The Lippman cyclone collected and separated the particles by size as recommended by the ACIGH [12]. This apparatus allowed measurement of the total dust, or total suspended particles (TSP), and the respirable fraction of dust (RF) concentration, measured in the rooms at 2 sampling stations. The pump was connected to an open-faced membrane-holder; for this purpose, membranes of 20 mm diameter nitro-cellulose were used, with a dust-spot of 14 mm and a pore size of $0.8 \mu\text{m}$.

The membranes were weighed before and after dust collection. The weighing took place in an environment with controlled humidity, using a micro-decimal balance (0.000001 g), following heat treatment in an oven at a temperature of $80-85^\circ\text{C}$. The sampling method was devised specifically to collect a quantity of dust on the membrane of $0.3-2 \text{ mg}$, corresponding to $2-12 \mu\text{g mm}^2$, so that the qualitative and quantitative measurements were performed correctly, as assessed in a previous study [13].

Samples and the other parameters were performed at 3 measurements points in each room, near the entrance door (1), in the middle of the room (2), at the end of the room (3) (Fig. 1).

The second dust measuring apparatus used was a Microair 5230 (HIAC ROYCO), designed to segregate particles into 6 size classes ($0.65-1.1 \mu\text{m}$; $1.1-2.1 \mu\text{m}$; $2.1-3.3 \mu\text{m}$; $3.3-4.7 \mu\text{m}$; $4.7-7 \mu\text{m}$; $7-7.5 \mu\text{m}$), and the concentration of each airborne particle size class was expressed in particles m^{-3} . The particle counts lasted 30 minutes with 50 cycles per count and 30 seconds for each cycle. The optical chamber was cleaned in the 6 second interval between counts; the airflow speed of the sample was $0.00047 \text{ m}^3\text{s}^{-1}$.

The coincidence error of the counter was less than 10% and the sizing error less than 5%, the counter covered the range of $0.3-25 \mu\text{m}$, in 8 user-defined channels. Monitor readings were taken at 15-day intervals, the first readings performed at day 0, 15, 30, 60 and 75 of the trial; 2 membranes were collected every 30 minutes during each sampling day and from each room to measure dust weight from the obtained 48 values measured by the instruments in 24 hours of sampling. From this, the mean value was calculated.

Microbial load – aerobic bacteria and fungal spores. Airborne bacteria, *Enterococcus faecium*, *Enterococcus faecalis*, Micrococcaceae (*Staphylococcus spp.*) and fungal spores were collected with a 6-stage Andersen sampler (Model 20-830 cut-point sizes: 9.0, 5.8, 4.7, 3.3, 2.1, 0.65, and $0.43 \mu\text{m}$) operating for 30 s to 4 min and located at a height of 1.7 m from the floor.

The Andersen 6-stage sampler is a cascade impactor with 400 holes per stage, drawing air at a flow rate of 28.3 l min^{-1} , and equipped with plastic Petri dishes containing 35 ml of the appropriate media and calibrated with plates in place.

Samples were performed at 3 measurements points in each room, near the entrance door (1), in the middle of the room (2), at the end of the room (3), see Figure 1.

The bacterial load of *Enterococci* was measured using Barnes medium, the plates incubated for 24 hours at 37°C , and the multiplied colonies – with colour varying from pink to violet – were isolated and typed according to their biochemical characteristics. The bacterial load of the Micrococcaceae was measured using Zebowitz medium, a selective solid medium for *Staphylococci*. Following sampling, the plates were incubated for 24 hours at 37°C ; the brown-black multiplied colonies were isolated and typed according to their biochemical characteristics.

Fungal spores were counted using Petri dishes containing a malt-based culture media (M_2 and M_5S_5) and incubated at 25°C and 35°C , respectively, for 5–7 days.

Statistical analysis. Data were submitted to analysis of variance using PROC GLM of SAS 9.2 [14] to study the effects of the fogging disinfectant procedure on piglets growth performance, ammonia, dust concentration, feed particle size class, aerobic bacteria and fungal spores.

RESULTS

The following sections present the results of the variance analysis; the model adopted to analyze data produced a minimum coefficient of determination of 0.54.

Piglets growth performance. A similar average number of dead piglets was recorded in the 2 rooms during the 2 phases of study, with a mean value of 12 dead piglets (4.2 %) in the

experimental room and 10 in the reference room (3.7%). Pre-weaning mortality, usually acceptable at 7%, is variable in swine farms, although recent data has indicated lower levels of pre-weaning mortality occurring in the summer and winter, with small peaks (12%) occurring in spring and autumn (NADIS).

At birth, the piglets' weight showed no significant difference between the 2 rooms for piglets from primiparous and pluriparous sows (Tab. 3).

Table 3. Mean weight (kg) of piglets reared in the 2 farrowing rooms

Day of age	Experimental room		Reference room	
	Mean weight (kg)	Mean number of lodged animals=284	Mean weight (kg)	Mean number of lodged animals=273
	Primiparous (15)	Pluriparous (15)	Primiparous (15)	Pluriparous (15)
0	1.420 ± 0.045	1.445 ± 0.028	1.390 ± 0.031	1.285 ± 0.067
15	4.2a ± 0.127	4.3a ± 0.141	4.1a ± 0.134	5b ± 0.312
30	7.5b ± 0.070	7.6b ± 0.410	6.9a ± 0.636	7.7b ± 0.288
75	26.7 ± 1.555	26.3 ± 0.565	26.6 ± 1.131	26.4 ± 0.424

a, b – data on the same row differ for p<0.05

On the last day of the trial, at 75 days of age, there was no significant difference in the average weight of the animals (approx. 26.5 kg) lodged in the 2 rooms.

Environmental parameters, temperature and relative humidity. The mean values and the standard deviation of temperature and relative humidity recorded in the 2 rooms are shown in Table 4. There was no significant difference for T and RH in the 2 rooms. Mean ventilation rates measured during ammonia and dust observation sampling period are reported, ventilation rate was similar in the 2 rooms (Tab. 5).

Ammonia concentration measurements. The mean values of ammonia concentrations during the observation periods are shown in Tab. 6.

Table 4. Mean values and standard deviation of temperature and relative humidity measured in 2 porcine farrowing-weaning room

	Variables	Minimum	Maximum	Mean ± SD
Experi-mental room	T (°C)	20.7	27.2	24.4 ± 3.2
	R.H. %	43.5	80.8	45.6 ± 28.8
	Ventilation rate (m ³ h ⁻¹)	815	3555	2873
Reference room	T (°C)	22	29.2	25.6 ± 3.6
	R. H. %	46.3	71.8	46.4 ± 22.8
	Ventilation rate (m ³ h ⁻¹)	800	3850	3011

Table 5. Mean ventilation rates (mg m⁻³) measured during ammonia and dust measurements in the 2 porcine farrowing-weaning rooms

Sampling time	Ventilation rate (m ³ h ⁻¹)	
	Reference room	Experimental room
0	3765	3727
15	3179	3225
30	2790	2603
60	2826	2700
75	2495	2110
Overall mean values	3011	2873

Table 6. Mean values of 24 four hours average ammonia concentration (mg m⁻³) in the 2 porcine farrowing-weaning rooms

Sampling time	Experimental room	Reference room
	Ammonia concentration (mg m ⁻³)	
0	0.56A	1.39B
15	2.79	2.79
30	6.97	6.97
60	11.84a	14.63b
75	22.89A	29.26B

(a, b) data on the same row differ for p<0.05;

(A, B) data on the same row differ for p<0.01

The air quality, from this point of view, at day 0, was significantly better in the reference room, which showed a lower ammonia concentration (1.39 vs. 0.56 mg m⁻³, p<0.01). On day 60, the mean ammonia concentration was 11.84 mg m⁻³ for the experimental room vs. 14.63 mg m⁻³ for the reference room (p<0.05).

At the fifth measurement (75 days of piglets' age), corresponding to the last days of piglets in the rooms, mean ammonia concentration in the experimental room reached the value of 22.89 mg m⁻³ vs. 29.26 mg m⁻³ in the reference room (p<0.01).

Dust concentration measurements. In both farrowing-weaning rooms, dust concentration, measured as TSP and RF, increased during the 75 days of observation (Tab. 7).

Table 7. Mean values of 24 hours average concentrations (mg m⁻³) of total suspended particles (TSP) and respirable fraction (RF) in the 2 porcine farrowing-weaning rooms

Sampling time	TSP mg m ⁻³		RF mg m ⁻³	
	d	Experimental room	Reference room	Experimental room
0	0.081	0.081	0.013	0.013
15	0.180a	0.310b	0.160	0.108
30	0.230A	0.745B	0.160a	0.375b
60	0.270A	2.970B	0.120A	1.050B
75	1.300A	3.200B	1.200a	1.630b

a, b – data on the same row differ for p<0.05

A, B – data on the same row differ for p<0.01

TSP – total dust or total suspended particles

RF – respirable fraction

Dust concentrations, intended as respirable fraction (RF) and total suspended particles (TSP), measured by the 2-stages Lippman selector, were similar in the 2 rooms at the beginning the trial; during the following weeks, however, the dust concentration was generally lower in the experimental room.

Both rooms showed the same value of 0.081 mg m⁻³ for TSP at day 0. At the following measurements, the TSP concentration was always lower in the experimental room – up to 1.3 mg m⁻³ vs. 3.2 mg m⁻³ for the reference room (p<0.01) at the last measurement.

In general, RF or the respirable fraction in the disinfected room was lower than in the reference room; at the end of the trial, the respirable fraction was 1.63 mg m⁻³ in the reference room and 1.2 mg m⁻³ in the experimental room (p<0.05).

The number of particles counted by the laser instrument Microair 5230 (HIAC ROYCO), transformed into logarithmic values, is shown in Figure 2.

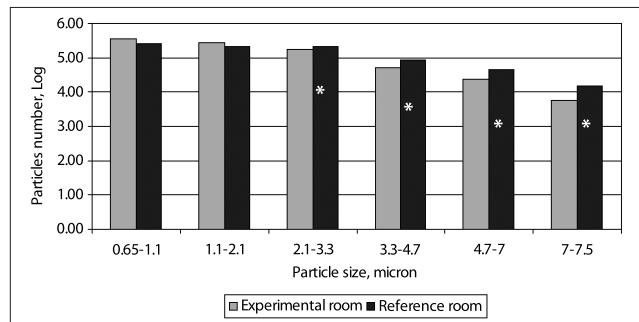


Figure 2. Mean number of dust particles sorted by size.

* data differ for p<0.05

In general, in both rooms, the dust class ranging from 0.65 µm – 1.1 µm presented the higher number of particles; the disinfectant fogging procedure reduced the number of particles larger than 2.1 µm, while the smaller particles, belonging to the aerodynamic classes ranging from 0.65–1.1 µm and from 1.1–2.1 µm remained unaffected by the treatment.

Aerial contaminants concentration. Results from the 6-stage phase Andersen separator are shown in Table 8, with mean values of CFU of the faecal enterococci, micrococci and fungal spores for particle size in the 2 rooms. In general, the variance analysis underlined a significant effect of dust particle size on CFU number for *E. faecium*, ($P \leq 0.0001$) and, *E. Faecalis*; in fact, the higher number of these bacteria was detected for the smallest particles with a size ranging from 0.65 µm – 1.1 µm.

Table 8. Mean concentrations (CFU m⁻³) of Enterococci, Micrococcaceae and fungal spores collected for the different dust particle size classes, in the 2 porcine farrowing-weaning rooms

Dust class particle size (µm)	<i>E. faecium</i> CFU m ⁻³		<i>E. faecalis</i> CFU m ⁻³		Micrococcaceae CFU m ⁻³		Fungal Spores CFU m ⁻³	
	Experimental room	Reference room	Experimental room	Reference room	Experimental room	Reference room	Experimental room	Reference room
0.65-1.1	120	124	11	6	50	42	1.2a	2b
1.1-2.1	67	21	3	2	50	16	0.06a	0.46b
2.1-3.3	80	28	5	3	45	31	0a	0.2b
3.3-4.7	44	23	3	2	20	16	0.26a	0.86b
4.7-7	25	7	2	1	13	15	0a	0.2b
7-7.5	1	2	0	0	3	10	0	0
TOTAL	338	205	24	14	180	130	15a	184b

(a, b) data differ for p<0.01;

No significant differences in the concentrations of *Enterococcus* spp. and Micrococcaceae in the air were found between the experimental and reference rooms.

Fungal spores were lowered by the treatment, with 15 total CFU for the experimental room vs. 184 total CFU of the reference room ($p < 0.01$). The finest particulate matter fractions (up to the dust class particles with size ranging from

4.7–7 µm) showed the highest fungal spores concentration in the reference room.

DISCUSSION

Animal performance and environmental conditions were similar in the 2 rooms, with no significant differences at the end of the trial.

Ammonia concentration increased during the trial in both rooms, depending on the increasing weight of the piglets, according to [15] the reduced ventilation rates following the decrease of outside temperature: at the end of the production cycle, in both rooms, ammonia level widely exceeded the value considered to be the most common and recommended threshold of 20 ppm, or 14 mg m⁻³ [16].

The reference unit showed a significantly higher ammonia concentration than that measured in the experimental unit throughout the observation periods: ammonia concentration reduction induced by fogging disinfectant treatment was estimated at 18 %, with overall mean values of 9.01 mg m⁻³ for the treated room vs. 11.01 mg m⁻³ for the reference room.

The difference in ammonia concentration between the 2 rooms, under similar environmental conditions and with a parity of lodged animals, can be explained by the disinfection procedure using the fogging system which acted as an aerial ammonia reducer, for the oxidizing effect of Virkon S [17].

In both farrowing rooms, ammonia TSP and RF increased during the 75 days of observation, and both dust fractions resulted in reduction by the fogging treatment. This is in agreement with a study conducted by Ellen et al. [18] who measured the impact of modifying relative humidity on dust levels in broiler houses. The study demonstrated that in houses fitted with fogging equipment, inhalable dust levels were reduced 13 and 22.5% during autumn and spring flocks, when the buildings were maintained at 75% RH, compared with control buildings. A slight immediate effect on the respirable dust was observed after fogging with pure water or water with rapeseed oil.

Analysis of particle size concentration showed that the number of particles larger than 2.1 µm resulted in a lowering by the disinfectant fogging treatment, while the smaller particles remained unaffected by the treatment.

Fungal spores were also reduced by the treatment, probably because of the oxidizing effect of Virkon S, which is more efficient in fungal spores reduction [17].

In fact, the peroxyomonosulfate principal component of Virkon has an oxidant effect on spores: spores have several hard coats that are very resistant to chemicals and physical stresses, such as heat, ultraviolet light, and temperature.

Fungal spores, considered an important etiological agent for allergic reactions, hay fever, rhinitis, asthma or pneumonitis [19], were significantly reduced by the disinfectant fogging treatment.

The finest particulate matter fractions showed the highest fungal spores concentration, probably associated with the higher number of particles in those classes, in agreement with research [20, 21] which showed that airborne microorganisms are easily adsorbed on the dust particle surface. Moreover, Omland [2] reported that the majority of spores for many fungi are in the respirable size range (<5 µm).

The bacterial load increased over time in both rooms and the treatment did not show any effect on microorganisms

reduction. The ineffectiveness of fogging treatment could be the result of the combination of a wide number of variables, such as inside temperature [22], relative humidity), air distribution [23] and animal activity [24], all of which are factors that in swine houses could affect the release of aerial contaminants, despite the performed disinfection fogging procedure.

CONCLUSIONS

In the presented research, the effects of a disinfectant fogging procedure on animals performance, air quality (dust concentration and ammonia), bacterial load and fungal spores concentration were evaluated.

In the case of dust reduction (particles larger than 2.1 μm and smaller than 7.5 μm), the efficacy of the treatment seemed linked more to the mechanical action of the spraying procedure than to a true anti-microbial action.

The reduction of ammonia (18%) and fungal spores in the experimental room, associated with finest particulate matter fractions, depended on the oxidizing effect of the disinfection procedure. In conclusion, this procedure improved air quality in the examined piggery, enhancing the health of both workers and animals at the same time.

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