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MORPHOLOGY, CHEMICAL COMPOSITION, AND BACTERIAL CONCENTRATION OF AIRBORNE PARTICULATE MATTER IN RABBIT FARMS

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Abstract: Livestock houses are major sources of airborne particulate matter (PM), which can originate from manure, feed, feathers, skin and bedding and may contain and transport microorganisms. Improved knowledge of particle size, morphology, chemical and microbiological composition of PM in livestock houses can help identify major sources of PM and contribute to the development of appropriate source-specific reduction techniques. In rabbit production systems, however, there is limited information on specific particle characteristics. The objective of this study was to characterise airborne PM in rabbit farms in terms of morphology, chemical compositions and bacterial concentration in different size fractions. Size-fractioned PM was sampled in the air of 2 rabbit farms, 1 for fattening rabbits and 1 for reproductive does, using a virtual cascade impactor, which simultaneously collected total suspended PM (TSP), PM10 and PM2.5 size fractions. Airborne PM samples were examined by light microscopy and scanning electron microscopy combined with energy dispersive X-ray analysis. Representative samples from potential sources of PM were also collected and examined. Additionally, a methodology to extract bacteria from the collected samples of airborne PM was developed to determine the bacterial concentration per PM size fraction. Results showed that airborne PM in rabbit farms is highly complex in particle morphology, especially in size. Broken skin flakes, disintegrated particles from feed or faecal material from mechanical fracture are the main sources of airborne PM in rabbit farms. Major elements found in rabbit airborne PM were S, Ca, Mg, Na and Cl. Bacterial concentrations ranged from 1.7×10^4 to 1.6×10^6 colony forming units (CFU)/m^3 (TSP); from 3.6×10^3 to 3.0×10^4 CFU/m^3 (PM10); and from 3.1×10^3 to 1.6×10^4 CFU/m^3 (PM2.5). Our results will improve the knowledge on essential particle characteristics necessary to understand PM's origin in rabbit farms and contribute to its reduction.

Key Words: Air quality, housing, bioaerosol, dust, rabbit, SEM-EDX.

INTRODUCTION

Livestock houses are major sources of airborne particulate matter (PM), which can originate from several sources: manure, feed, feathers, skin and bedding (Donham *et al.*, 1986; Cambra-López *et al.*, 2011a). The heterogeneous nature of PM in livestock houses comprises particles of different morphology and chemical composition (Cambra-López *et al.*, 2010). Moreover, particle size is one of the most relevant properties related to the potential health and environmental hazards of PM (Harrison and Yin, 2000). In livestock environments, airborne PM includes size fractions ranging from fine (PM which passes through a size-selective inlet with a 50% efficiency cut-off at 2.5 µm, PM2.5), coarse (PM which passes through a size-selective inlet with a 50% efficiency cut-off at 10 µm, PM10), and total suspended particles (all airborne particles, TSP). Furthermore, particle size and morphology are very closely related to lung deposition

(Zhang, 2004). Consequently, high concentrations of PM can cause detrimental effects on animal performance and efficiency (Donham and Leininger, 1984; Donham, 1991; Al Homidan and Robertson, 2003) and the health and welfare of farmers (Andersen *et al.*, 2004; Donham *et al.*, 1984). Emitted PM can also have detrimental effects on the environment (Grantz *et al.*, 2003).

The morphology and chemical composition of PM depends on livestock species and housing systems. In poultry, Cambra-López *et al.* (2011a) reported that the most abundant sources of airborne PM are feathers and uric acid crystals; whereas in pigs the most abundant sources are manure and pig's skin. In addition, PM can contain and transport microorganisms (fungi, viruses, bacteria, toxins and allergens), some of them pathogenic (Bakutis *et al.*, 2004, Adell *et al.*, 2011) which can cause direct harm to humans and animals. In rabbit production systems, however, there is limited information on specific particle characteristics such as morphology (i.e. shape, size and texture), chemical composition, and microbiological components of PM.

Improved knowledge of particle size, morphology, chemical and microbiological composition of PM in livestock houses can help to identify major sources of PM. The best approach to reduce PM in and from livestock houses seems to be to prevent it from being generated directly at its source. Consequently, the characterisation of PM in livestock houses is essential to develop suitable reduction techniques. This better understanding would contribute to the development of efficient and practical source-specific reduction techniques to comply with European thresholds set in air quality regulations (Directive 1999/30/EC and Directive 2008/50/EC) and help protect the environment and human and animal health and welfare in and around rabbit farms.

The aim of this study was to characterise airborne PM in rabbit farms in terms of morphology, chemical compositions and bacterial concentration in different size fractions. Our results will improve the knowledge on essential particle characteristics necessary to understand PM's origin in rabbit farms and contribute to its reduction.

MATERIALS AND METHODS

Housing and animals

Two rabbit farms were surveyed in this study: one rearing fattening rabbits, and another rearing reproductive does. Animals were reared in cages in both farms. Manure was accumulated in pits below the cages for 3-4 wk. Both farms were located in the region of Valencia (East of Spain) and surveyed during summer.

Average indoor temperatures in the surveyed farms were 27.8 and 19.7°C for fattening rabbits and reproductive does, respectively. Regarding relative humidity, average values inside both buildings were 66.5 for fattening rabbits and 61.4% for reproductive does. Outdoors, temperature was 22.7 for fattening rabbits and 15.6°C for reproductive does and relative humidity was 62.2 for fattening rabbits and 58.6% for reproductive does. Table 1 describes both surveyed farms in terms of housing and animals.

Table 1: Description of the surveyed rabbit farms.

	Fattening rabbits	Reproductive does
Length×width (m)	30×6	45×7
Animal places	2100	400
Feed distribution	Manually distributed pellets	Manually distributed pellets
Ventilation	Tunnel mechanical, 2 fans	Tunnel mechanical, 1 fan

Particulate matter sampling

To characterise PM in rabbit farms, airborne PM was first sampled on each farm. Secondly, additional samples from potential known sources of PM were collected and examined to compare airborne samples against a reference of each PM sources.

Airborne PM sampling: A virtual cascade impactor (RespiCon, Helmunt Hund GmbH, Wetzlar, Germany) was used on each farm to sample PM2.5, PM10 and TSP in the air. Each PM size fraction was collected onto separate filters. Two types of filters were used: glass fibre filters (37 mm Ø, Helmut Hund, Wetzlar, Germany), for chemical, morphological and bacterial concentration analysis; and polycarbonate filters (37 mm Ø, 5 μm pore size), to examine PM characteristics in greater depth and confirm previous results obtained using glass fibre filters. Portable pumps (Genie VSS5, Buck Inc, U.S.) were used to draw air through each virtual cascade impactor at a constant flow of 3.11 L/min.

Sampling was conducted inside each farm, in the centre of the building, at 1.5 m height. Sampling frequency and time were adjusted to obtain sufficient particles for morphological and chemical composition examinations, on the one hand, and bacterial concentration analysis on the other. No gravimetric analyses were subsequently performed with filter samples. Samples used for morphological and chemical composition analyses were collected weekly, during 5 wk for fattening rabbits and 2 wk for reproductive doe buildings. Sampling duration was 6 d. Samples used for bacterial concentration examinations were collected twice in each facility. Sampling duration was 15 min, to minimise dehydration of bacteria. In this case, the virtual cascade impactor was disinfected with 96% alcohol prior to sampling and sterile glass fibre filters were used. After sampling, filters were transported to the laboratory under refrigeration (4°C).

Sampling for known sources of PM: Representative samples from potential sources of PM were obtained by randomly sampling at different locations in each building for feed, manure, hair, and powdered disinfectants normally used in rabbit farms (calcium superphosphate and sulphur). Composite samples were collected directly from farm surfaces, avoiding contamination among them. Each sample was then homogenised in the laboratory to achieve a uniform sample, then dried in the oven for 12 h at 70°C. Dried samples were crushed manually in a mortar.

To obtain size-segregated PM samples from the different known sources, a dust generator was used to aerosolise PM. The dust generator consisted of a stainless steel cylinder of 20 cm diameter and 30 cm height with an airtight lid, which had a mechanical agitation system and rotating blades at the end. The aerosolisation process of potential PM sources was conducted following the methodology and set-up described in Cambra-López *et al.* (2011b). The mass of sample and the dust generation time were adjusted depending on the sample. Approximately 40 g of feed, 3 g of manure, 0.4 g of hair, 1.2 g of sulphur, and 1 g of calcium superphosphate were placed in the agitation system. Sampling time varied between 1 min (sulphur and calcium superphosphate), 2 min (manure), 2 h (hair) and 12 h (feed). The PM generated during aerosolisation was collected in TSP, PM10 and PM2.5 size fractions, using a virtual cascade impactor (RespiCon, Helmunt Hund GmbH, Wetzlar, Germany) and a portable pump, the same as for airborne PM sampling, using polycarbonate filters.

Particulate matter characterisation

Morphology: Particle morphology was studied using 2 microscopic techniques: light microscopy (LM) and scanning electron microscopy (SEM).

Major PM components in airborne PM collected on glass fibre filters were qualitatively and quantitatively analysed using LM. Qualitative analysis was conducted with direct observations using a Nikon Eclipse E400 microscope at 10× and 20× magnification, and photomicrographs were taken

with a Nikon Ds-5M Camera, coupled to the microscope. A representative area of the glass fibre filter collected in the air of each farm was cut and mounted on a glass slide. At least 4 views (spots) per filter were examined. The different components identified in PM were described in terms of their size and morphology. Iodine (dilution 1:10 of iodine in distilled water) was used to stain starch granules and identify feed particles, by directly pipetting 1 to 3 mL of dilution onto the filter, following Donham *et al.* (1986). Quantitative analysis of the different components found in the airborne PM fractions was also performed. The PM components were counted in each examined view per filter.

Furthermore, samples of airborne PM collected on polycarbonate filters were analysed for particle morphology per size fraction using a high-resolution SEM (JEOL, JSM-5410, Tokyo, Japan). The SEM was used to support and complete LM analysis. The main advantages of using SEM were viewing particles at higher magnifications than using LM. Moreover, SEM was also used to morphologically examine samples from known sources of PM generated in the laboratory using the dust generator.

A small section (approximately 1 cm^2) of each polycarbonate filter from each size of fractions was cut and mounted on a 12 mm carbon stub with a double-sided carbon adhesive tape. Each sample was then coated with carbon using a vacuum evaporator to create a coating conductive to the SEM electron beam. Photomicrographs of each field of view were taken at varying magnifications ranging from 600× to 2500×.

As regards morphology using LM and SEM, particle components were identified compared to published photographs of known particles (McCrone, 1992; Cambra-López *et al.*, 2011b). Particle types were qualitatively analysed and morphologically described in terms of shape (rounded, spherical, fibrous, flake, angular, aggregate, irregular, flattened, long-thin), surface (layered, smoothed, cracked), edges and borders (sharpness), texture (smooth, grape-like, and rough), and opacity, amongst others (McCrone, 1992; NIST, 2010).

Chemical composition: Samples of airborne PM collected on glass fibre filters were analysed for particle chemical composition, per size fraction, using high resolution SEM (JEOL, JSM-5410) combined with energy-dispersive X-ray analysis (EDX, Link Tetra Oxford Analyzer, Oxfordshire, U.K.). Preparation of samples was the same as for morphological analysis using SEM with polycarbonate filters. The SEM/EDX was conducted manually to obtain particle-by-particle element chemical composition.

Elements with atomic number ≥11 (sodium) were detected from the element X-ray spectra. At least 3 fields of view (spots) per filter sample were analysed. On each analysed field, the elemental spectra of every particle found were analysed. For quantitative element analyses, EDX spectrograms were recorded and analysed using Oxford INCA Software (Oxford Instruments, Abingdon, U.K.).

The effect of PM size fraction on element chemical composition in the analysed particles was tested with one-way ANOVA using SAS (2001), with size fraction as source of variance, and the individual particle element composition as the experimental unit in the ANOVA analyses.

Bacterial concentration: A methodology to extract bacteria from the collected samples of airborne PM on glass fibre filters was developed to determine the microbiological content of the different PM size fraction.

Each sample collected on glass fibre filters was eluted in 25 mL of Nutrient Broth, adding 0.05% Tween 20, and shaken for 90 min at 200 rpm at room temperature. One-mL samples were transferred from the suspension on duplicate plates directly onto Compact Dry TC (Hyserve GmbH & Co., Uffing, Germany). Plates were incubated at 37°C for 72 h under aerobic conditions. Airborne concentrations of aerobic bacteria were determined by multiplying the colony forming units (CFU) by the eluted volume, and divided by the volume plated (1 mL) and the volume of sampled air.

RESULTS

*Particulate matter characterisation*

Morphology: Qualitative LM analysis of the different components found in the PM from fattening rabbit and reproductive does revealed that PM from rabbit farms was highly diverse and comprised particles heterogeneous in size, morphology and origin. Seven different particle components were identified in PM using LM: feed, faecal material, dander and skin cells, hair, mould and fungus, insect parts and sulphur particles. Some of them previously described in Cambra-López and Torres (2008).

Starch granules from feed appeared as round, smooth and flattened particles. These could be stained with iodine, turning into a violet blue colour. Feed particles were highly agglomerated, but individual particles ranged from 3 to 30 µm in diameter (Figure 1a). Faecal particles were irregular in shape and size, and included heterogeneous components such as undigested feed residues. Faecal particles showed rounded edges in some cases, and acute edges in others. Particles were quite rough, showing a dark yellow to brown colour (Figure 1b). Generally, these were darker in colour than feed particles, although discrimination between them was complicated. Dander and skin particles were flat, smooth, and transparent compared with other components in PM, and irregular in size. Particles from skin showed a relatively platy or flake-like morphology, with folded up edges (Figure 1c). Rabbit hair was easily detected and identified as long-thin structures, generally 5 to 30 µm in diameter, with a central canal characterised by a ladder-like chain of patches, similar to a string of pearls (Figure 1d). Spores from mould and fungal conidia were also identified. Spores were transparent, colourless and smooth with oval bodies 3 to 5 µm in diameter (Figure 1e). Conidia were transparent, dark brown and walled structures, forming 2 to 4 chambers of approximately 5 to 20 µm wide and 12 to 40 µm long (Figure 1f). Hyphae were also identified as individual fibres, transparent, colourless or yellowish walled structures. Insect parts such as insect wings were easily identified (Figure 1g). Sulphur particles were round, smooth, yellow in colour, and varied in size from 20 to 100 µm (Figure 1h).

Besides the qualitative analysis of PM components and their identification through LM, a quantitative analysis of these components was also performed. Results from the quantitative analysis are shown in Table 2. This analysis could only be conducted in TSP and PM10 fractions due to the limitations in magnification of LM in the PM2.5 fraction, together with its small size. Hence, Table 2 shows results for just one sample in PM2.5, where the high value corresponding to the "Others" component (62%) reveals the difficulty of such analysis in this fraction. The "Others" fraction represented unclassified particles or fragments of any of the 7 identified components not easily distinguished using LM by their shape, colour or size. However, to a certain extent, feed, faecal material and skin particles were identified.

Quantitative analysis using LM showed that feed components and faecal material composed the bulk of the collected particles in all fractions, ranging from 25 to 63% for feed and from 11 to 22% for faecal material. The counted number of particles from feed increased from PM2.5 to TSP. The counted number of particles from faeces remained constant in the 3 fractions. The high value attributable to the "Others" component in PM2.5 fraction, however, could alter these results. The rest of the components were easily counted due to their differential morphologies and were found to a lesser extent, with percentages generally below 13% in all cases. Sulphur particles were only present in the reproductive doe farm, and ranged from 9 to 13% in PM10 and TSP fractions.
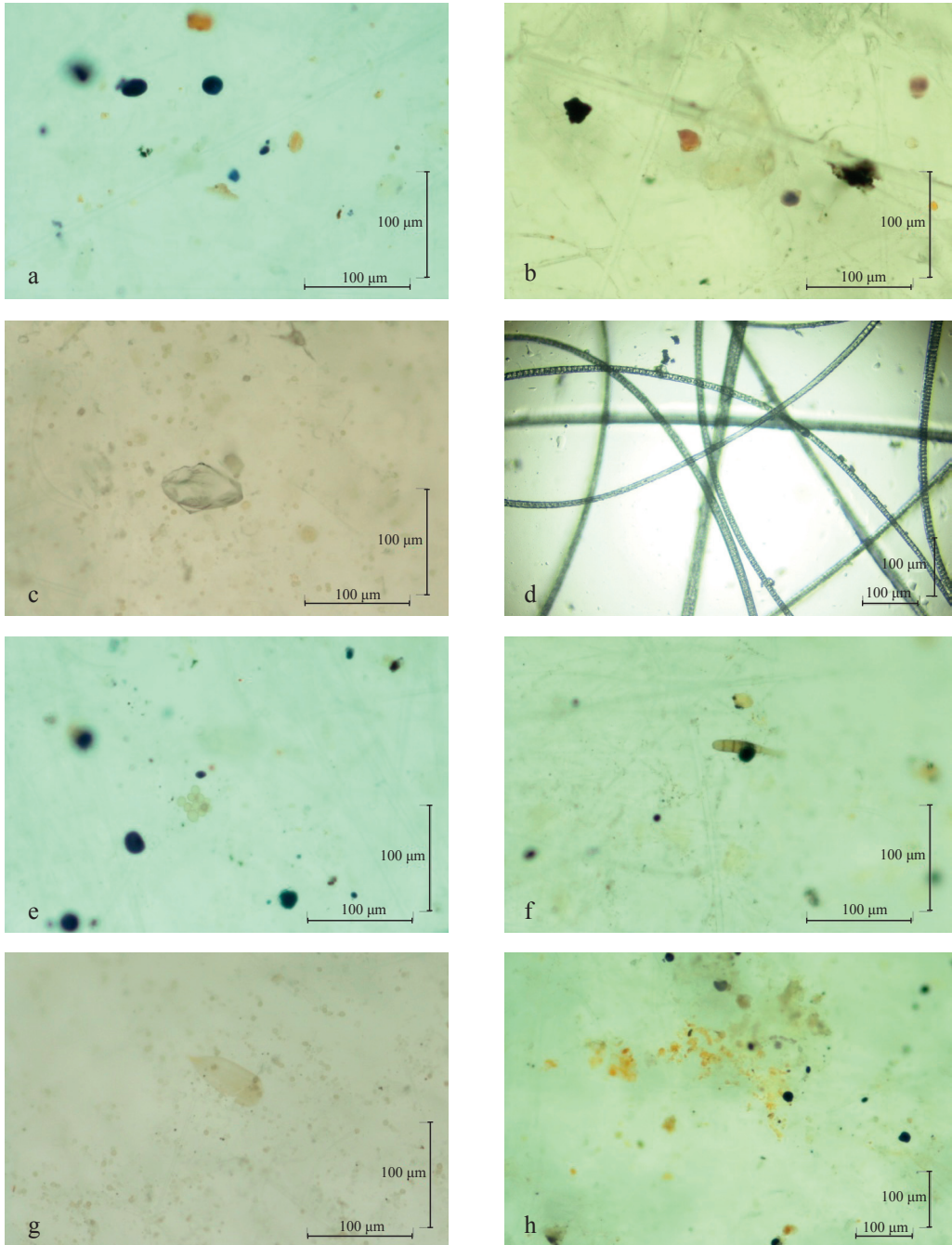
Figure 1: Particulate matter components viewed using light microscopy (10× and 20×) on airborne samples collected on glass fibre filters in fattening rabbit and reproductive does. Particles from feed (a), faecal material (b), skin (c), hair (d), fungal spores (e), conidia (f), insect wings (g) and sulphur (h). Scale bar 100 µm.

Table 2: Number of particles from the different components identified through light microscopy in the collected particle matter (PM) from fattening rabbit and reproductive does, expressed as average relative percentage (%) and standard deviation.

| PM size[1]	Components	Fattening rabbits	Reproductive does
PM2.5[2]	Feed	25.2	ND
	Faecal material	11.1	ND
	Skin	1.9	ND
	Others	61.9	ND
	Total counted particles	163	-
PM10	Feed	53.4±6.4	37.5±11.4
	Faecal material	21.7±10.8	23.2±21.5
	Skin	2.1±0.7	13.1±16.1
	Hair	0.1±0.3	0.2±0.3
	Microorganisms	0	3.0±1.9
	Sulphur	0	12.8±18.1
	Others	22.7±15.1	10.1±0.5
	Total counted particles	920	371
TSP	Feed	62.9±7.1	50.8±5.3
	Faecal material	18.4±6.9	17.9±15.5
	Skin	3.0±1.6	12.3±11.5
	Hair	0.3±0.5	1.4±0.2
	Microorganisms	0.1±0.2	1.4±0.4
	Sulphur	0	8.6±12.1
	Others	15.3±8.9	7.7±2.3
	Total counted particles	768	353

ND= No data. [1]PM size: PM which passes through a size-selective inlet with a 50% efficiency cut-off at 2.5 (PM2.5) and 10 μm (PM10). TSP, all airbone particles. [2]Only one sample was observed.

Airborne PM samples collected on polycarbonate filters and examined under SEM are shown in Figure 2. This figure illustrates the different PM components and confirms their presence and quantities calculated using LM. In fattening rabbits, Figure 2 shows heterogeneous particles, which could be grouped into four particle types: feed, faecal particles, dander, and calcium superphosphate (as explained before, known to be used on fattening rabbit farms as disinfectant). Differences in the abundance of these components between fractions are evident from this figure. In PM2.5, most particles appeared as small bright particles probably from feed, whereas in PM10 and TSP, large skin flakes and irregular layered PM were highly abundant.

Specific individual particle components generated from known sources viewed by SEM are shown in Figure 3. Morphological structures ranging from transparent flake-like bent skin cells or rabbit dander (Figure 3a), irregular angular and layered faecal particles (Figure 3b), round and small particles from feed (Figure 3c), aggregates of calcium superphosphate particles (Figure 3d), spore-like bioaerosol, presumably conidia from fungus (Figure 3e), and long-thin pointed particles from hair (Figure 3f) were found.

Chemical composition: Average element chemical composition is presented in Table 3, showing differences in element percentages among size fractions.

Glass fibre filters showed presence of Na, Al, Si, K, Ca, Zn and Ba. In addition to the elements present in the blank filter (glass fibre filter), high contents of S and Ca were identified in all size fractions. Cl was more abundant in PM10 and TSP fraction compared with PM2.5; whereas other
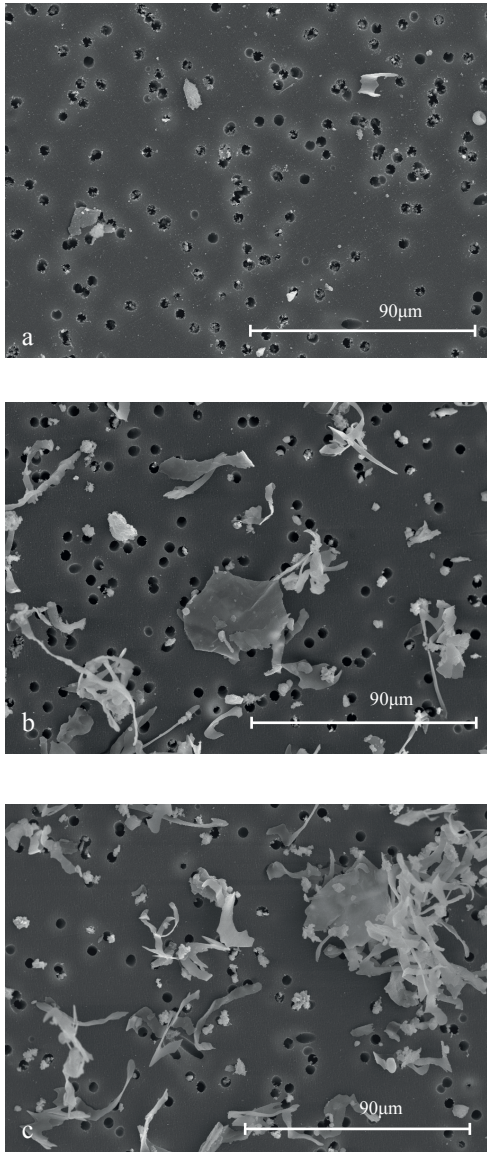
Figure 2: Particulate matter airborne samples collected on polycarbonate filters viewed using scanning electron microscopy (600×), from PM2.5 (a), PM10 (b) and TSP (c) samples from fattening rabbit farm. Note: 5 µm diameter filter pores are shown as round dark holes. Scale bar 90 µm. Particle size (PM) which passes through a size-selective inlet with a 50% efficiency cut-off at 2.5 (PM2.5) and 10 µm (PM10). TSP, all airbone particles.

elements such as Mg and P were the most abundant in TSP, and Fe was the most abundant in PM2.5. From the ANOVA analysis, it was observed how the differences in the average values of the most abundant elements (Na, Mg, P, S, Cl, K, Ca, Zn and Ba) were significantly different in 1 or 2 size fractions. Overall, major elements found in rabbit airborne PM were S, Ca, Mg, Na and Cl.

Bacterial concentration: Table 4 shows the results for the average airborne bacteria concentrations in CFU per m^3 of air from the samples collected in fattening rabbit and reproductive doe farms. Average CFU in the air were higher in TSP compared with other fractions, and overall ranged from 1.7×10^4 to 1.6×10^6 CFU/m^3. Average CFU in PM10 ranged from 3.6×10^3 to 3.0×10^4 CFU/m^3, and from 3.1×10^3 to 1.6×10^4 CFU/m^3 in PM2.5.

DISCUSSION

The results presented herein contribute to improving the knowledge on airborne PM in rabbit farms in terms of particle morphology, chemical compositions and bacterial concentrations in different size fractions. Particle characterisation revealed high particle diversity in rabbit PM. Although most particles were biological in nature, quantitative analysis using LM showed that feed components and faecal material composed the bulk of the airborne particles in PM2.5, PM10 and TSP size fractions, ranging from 25 to 63% for feed and from 11 to 22% for faecal material.

Previous studies in pigs (Donham *et al.*, 1986; Heber *et al.*, 1988; Feddes *et al.*, 1992) identified feed as predominant components in TSP and in particles larger than 10 µm in diameter. In poultry and pigs, Cambra-López *et al.* (2011a) found a higher proportion of particles from faecal material in PM10 and PM2.5 than in our study. Perhaps the nature of rabbit's hard faeces, which are highly compressed and have a mucin cover (Sirotek *et al.*, 2003), could probably explain such

Table 3: Average element composition (%) and standard deviation of the different particle matter (PM) size fractions, including blank filter, and significance level of average values among fractions (n=159).

	Blank filter	PM2.5	PM10	TSP	*P*-value
Na	6.7±0.4	5.3±2.1[a]	7.8±4.9[b]	6.4±3.9[ab]	<0.05
Mg	0	0.3±0.5[a]	0.9±0.8[b]	1.3±1.3[c]	<0.001
Al	4.9±0.3	5.0±2.8	4.4±3.1	4.0±1.8	NS
Si	49.6±1.6	39.3±12.8	36.4±12.8	40.8±17.2	NS
P	0	0[a]	0.3±0.9[a]	1.5±5.1[b]	<0.10
S	0.1±0.2	7.7±8.4[b]	5.0±6.7[a]	4.2±5.3[a]	<0.10
Cl	0	0.1±0.3[a]	4.3±5.6[b]	3.1±5.3[b]	<0.001
K	6.8±0.1	5.8±1.9[a]	6.4±1.7[ab]	7.2±4.6[b]	<0.10
Ca	4.5±0.4	14.5±13.3[b]	10.6±13.3[ab]	17.8±16.9[a]	<0.10
Mn	0	0.0±0.1	0	0.0±0.1	NS
Fe	0.2±0.3	2.3±8.3	0.7±1.5	0.4±0.9	NS
Ti	0	0.3±2.1	0.1±0.4	0.0±0.1	NS
Zn	12.4±1.1	8.2±4.7[b]	10.5±7.3[c]	5.4±2.9[a]	<0.001
Ba	14.9±1.1	11.1±7.1[b]	11.8±5.7[b]	7.8±4.2[a]	<0.05
I	0	0.0±0.3	0.9±3.2	0	NS
Ce	0	0.1±0.5	0	0	NS
Cu	0	0.0±0.2	0	0	NS

[a,b]Averages within a row with different superscripts differ significantly (*P*<0.10). NS: *P*>0.10. PM which passes through a size-selective inlet with a 50% efficiency cut-off at 2.5 (PM2.5) and 10 μm (PM10). TSP, all airbone particles.

differences. Airborne PM in rabbit houses showed a high relative contribution of feed and rabbit skin and hair, compared with other species. Moreover, our results showed a high complexity in particle morphology (especially in size, which ranged from a few μm to 90-100 μm) in the examined PM samples. This indicates that source contributions could vary when expressed in particle mass rather than in particle numbers as reported in Cambra López *et al.* (2011a). In fact, large particles from skin as shown in Figure 2 could gain relative importance when expressed in particle mass.

Both LM and SEM were used in this study to discriminate among particle components and types (i.e. sources). When using LM, iodine was used to stain starch granules and differentiate feed from the other sources. Undigested feed components from feed found in faecal material could also be stained with iodine. Furthermore, faecal particles were difficult to distinguish, especially in PM2.5 fraction, and could in some cases be confused with skin or feed. For this reason, the proportion of feed might have been slightly overestimated in our results using LM. The use of another stain different from iodine to differentiate between feed or faecal material such as undigested feed particles found in faeces could help in the identification of these PM components in the smaller size fractions when using LM. Nile blue sulphate stain has been used before for this purpose (Donham *et al.*, 1986). Nevertheless, to overcome LM limitations,

Table 4: Average airborne bacterial concentrations and standard deviation in colony forming units (CFU) per m^3 in fattening rabbit and reproductive does in different particle matter (PM) size fractions and standard deviation (n=2).

	PM2.5	PM10	TSP
Fattening rabbits	4.2×10^3±2.3×10^3	7.9×10^3±6.1×10^3	4.1×10^5±5.6×10^5
Reproductive does	1.2×10^4±1.1×10^3	1.9×10^4±1.2×10^4	9.4×10^4±5.1×10^4

PM which passes through a size-selective inlet with a 50% efficiency cut-off at 2.5 (PM2.5) and 10 μm (PM10). TSP, all airbone particles.
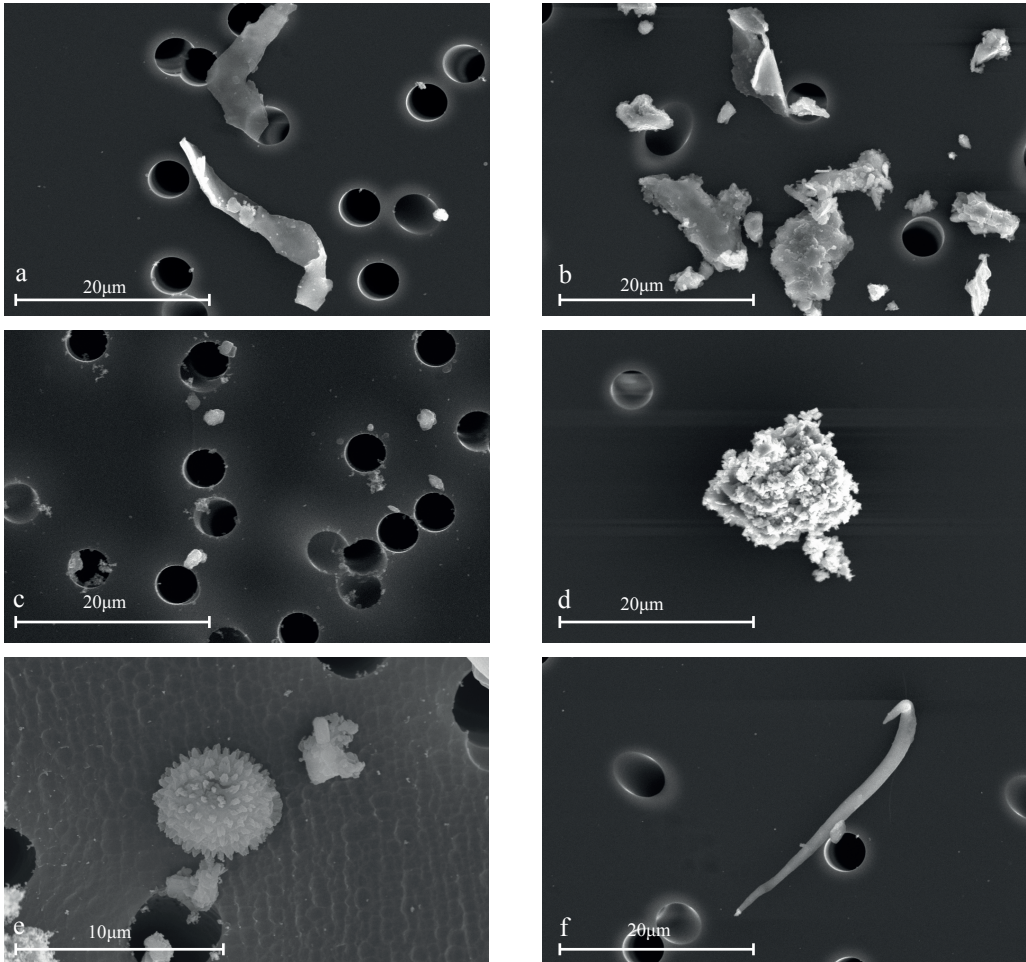
Figure 3: Examples of scanning electron microscope photomicrographs from particle matter generated in the laboratory from known sources collected in fattening rabbits and reproductive does on polycarbonate filters showing rabbit dander (a), layered faecal particles (b), feed (c), calcium superphosphate particles (d), bioaerosol (e) and hair particles (f). Note 5 µm diameter filter pores shown as round dark holes. Scale bar 20 µm, except for Figure 3e, equal to 10 µm.

further SEM analysis is encouraged. In fact, SEM analysis in this study was used to examine PM characteristics more closely and to support and complete LM analysis. The SEM analysis revealed that particles from skin, faeces and feed were abundant in TSP and PM10 fractions, whereas particles from feed and, to a lesser extent, from skin, were the most abundant in PM2.5. These data provide valuable information, especially as regards fine PM2.5, although further examinations using SEM are needed to acquire additional data on particle characteristics in rabbit farms under different housing and environmental conditions other than those in this study.

Our results indicate that most particles were characterised as fragmentation-type particles, with irregular and acute edges (broken skin flakes, disintegrated particles from feed or faecal material from mechanical fracture). These results are in agreement with the results obtained from analysis

of the farm activities influencing PM generation (Adell *et al.*, 2012). These authors reported that mechanical activities such as feeding, sweeping and cleaning the cages by burning hair are major PM-generating activities. Crushing of feed particles during feed distribution could explain the high contribution of feed particles found in airborne PM. Whether the rest of activities would result in the generation of faecal material, rabbit skin and hair would be a matter of discussion, but it could be expected that skin debris would be released through animal manipulation and other farm activities. Besides mechanical fragmentation of particles, a variety of biological structures such as spores were identified, indicating that fungal spores might be abundant in the air in rabbit farms.

Major elements found in rabbit PM were S, Ca, Mg, Na and Cl. These elements were similar to those reported by Aarnink *et al.* (2004) and Schneider *et al.* (2001) in airborne PM in pigs, and by Cambra-López *et al.* (2011b) in poultry, except for certain elements such as Ca (found in a greater extent in this study) and only small amounts of P. The higher content of Ca could be attributable to the use of calcium superphosphate powder in fattening rabbit farm.

Overall, the analytical methods used to characterise PM in this study, based on microscopic techniques, can supply valuable but limited data on particle or source chemical composition and morphological characteristics. To further identify and quantify source contributions, the use of source apportionment models is encouraged (Watson *et al.*, 2002). Source apportionment models would allow quantitative and comparable estimations of source contributions of PM, between and within livestock categories.

As regards the bacterial concentrations in airborne PM, our results were higher than those reported by Navarotto *et al.* (1995) and Duan *et al.* (2006) for rabbit farms. The findings of this study are similar to those observed by Seedorf *et al.* (1998) for cows, pig and poultry houses and Ribikauskas *et al.* (2010) for rabbit house. Although filtration samplers are not recommended for microbial bioaerosol sampling because of desiccation stresses that occur as air flows through the filters (Crook, 1996), it is a commonly used technique (Thorne *et al.*, 1992) and our findings indicate that airborne bacteria concentrations in rabbit farms are comparable with those in other livestock species. The values observed in airborne bacteria in rabbit farms suggest further research to investigate the presence and levels of infective airborne pathogens would be useful.

CONCLUSIONS

Airborne PM in rabbit farms is highly complex in particle morphology, especially in size, revealing high diversity in particle components and types (i.e. sources). Particle size ranged from a few μm to 90-100 μm and most PM showed fragmentation type particles with irregular and acute edges.

Broken skin flakes, disintegrated particles from feed and faecal material from mechanical fracture are the main sources of airborne PM in rabbit farms. Major elements found in rabbit airborne PM were S, Ca, Mg, Na and Cl. Further research is needed to obtain quantitative and comparable estimations of source contributions of PM, between and within livestock categories using source apportionment models.

Average CFU in the air ranged from 1.7×10^4 to 1.6×10^6 CFU/m^3 in TSP; from 3.6×10^3 to 3.0×10^4 CFU/m^3 in PM10; and from 3.1×10^3 to 1.6×10^4 CFU/m^3 in PM2.5. The existence of infective airborne pathogens in the air in rabbit farms, however, is still unknown.

Our results will improve the knowledge on airborne PM in rabbit farms in terms of morphology, chemical compositions and bacterial concentrations in different size fractions, necessary to understand PM's origin in rabbit farms and to propose adequate source-specific reduction techniques.

REFERENCES

Aarnink A.J.A., Stockhofe-Zurwieden N., Wagemans M.J.M. 2004. Dust in different housing systems for growing-finishing pigs. *In Proc.: Engineering the Future. AgEng 2004. Leuven, Belgium. Session 22.*

Adell E., Moset V., Zhao Y., Cerisuelo A., Cambra-López M. 2011. Concentración, distribución espacial y por tamaño de bacterias aerobias mesófilas en el aire de granjas de broilers. *ITEA-Inf. Tec. Econ. Ag., 107: 77-93.*

Adell E., Estellés F., Torres A.G., Cambra-López M. 2012. Particulate matter concentrations and emissions in rabbit farms. *World Rabbit Sci., 20: 1-11. doi:10.4995/wrs.2012.1035*

Al Homidan A., Robertson J.F. 2003. Effect of litter type and stocking density on ammonia, dust concentrations and broiler performance. *Brit. Poultry Sci., 44: S7-S8.*

Andersen C.I., Von Essen S.G., Smith L.M., Spencer J., Jolie R., Donham K.J. 2004. Respiratory symptoms and airway obstruction in swine veterinarians: A persistent problem. *Am. J. Ind. Med., 46: 386-392. doi:10.1002/ajim.20080*

Bakutis B., Monstviliene E., Januskeviciene G. 2004. Analyses of airborne contamination with bacteria, endotoxins and dust in livestock barns and poultry houses. *Acta Vet. Brno, 73: 283-289. doi:10.2754/avb200473020283*

Cambra-López M., Torres A.G. 2008. An approach to source apportionment of dust in animal houses: the case of rabbit rearing facilities. *In Proc: International Conference of Agricultural Engineering. CIGR 2008. Iguassu Falls, Brasil.*

Cambra-López M., Aarnink A.J.A., Zhao Y., Calvet S., Torres A.G. 2010. Airborne particulate matter from livestock production systems: A review of an air pollution problem. *Environ. Pollut., 58: 1-17. doi:10.1016/j.envpol.2009.07.011*

Cambra-López M., Hermosilla T., Lai H.T.L., Aarnink A.J.A., Ogink N.W.M. 2011a. Particulate matter emitted from poultry and pig houses: Source Identification and Quantification. *T. ASABE, 54: 629-642.*

Cambra-López M., Torres A.G., Aarnink A.J.A., Ogink N.W.M. 2011b. Source analysis of fine and coarse particulate matter from livestock houses. *Atmos. Environ., 45: 694-707. doi:10.1016/j.atmosenv.2010.10.018*

Crook B. 1996. Review: Methods of monitoring for process micro-organisms in biotechnology. *Ann. Occup. Hyg., 40: 245-260. doi:10.1093/annhyg/40.3.245*

Donham K.J. 1991. Association of environmental air contaminants with disease and productivity in swine. *Am. J. Vet. Res., 52: 1723-1730.*

Donham K.J., Leininger J.R. 1984. Animal studies of potential chronic lung-disease of workers in swine confinement buildings. *Am. J. Vet. Res.,* 45: 926-931.

Donham K.J., Zavala D.C., Merchant J.A. 1984. Respiratory symptoms and lung function among workers in swine confinement buildings: a cross-sectional epidemiological study. *Arch. Environ. Health, 39: 96-101.*

Donham K.J., Scallon L.J., Popendorf W., Treuhaft M.W., Roberts R.C. 1986. Characterization of dusts collected from swine confinement buildings. *Am. Ind. Hyg. Assoc. J., 47: 404-410. doi:10.1080/15298668691389955*

Duan H.Y., Chai T.J., Muller W., Zucker B.A. 2006. Concentration of airborne endotoxins and airborne bacteria in Chinese rabbit houses. *Berl. Mun. Tierarztl., 119: 40-44.*

Feddes J.J.R., Cook H., Zuidhof M.J. 1992. Characterization of airborne dust particles in turkey housing. *Can. Agr. Eng., 34: 273-280.*

Grantz D.A., Garner J.H.B., Johnson D.W. 2003. Ecological effects of particulate matter. *Environ. Int., 29: 213-239. doi:10.1016/S0160-4120(02)00181-2*

Harrison R.M., Yin J.X. 2000. Particulate matter in the atmosphere: which particle properties are important for its effects on health? *Sci. Total Environ., 249: 85-101. doi:10.1016/S0048-9697(99)00513-6*

Heber A.J., Stroik M., Faubion J.M., Willard L.H. 1988. Size distribution and identification of aerial dust particles in swine finishing buildings. *T. ASAE, 31: 882-887.*

McCrone Associates Inc. 2007. McCrone Atlas of microscopic particles. *Available at: http://www.mccroneatlas.com. Accesed January 2011.*

Navarotto P., Crimella C., Luzi F., Guarino M., Heinzl E., Dragoni I., Papa A., Massola A. 1995. Air-quality in the environment of intensively reared fattening rabbits. *Farm Build. Progr., 28-32.*

NIST. 2010. Particle morphology glossary. Glossary of morphology terms. *U.S. National Institute of Standards and Technology. Available at : http://www.nist.com. Accesed January 2011.*

Ribikauskas V., Ribikauskiene D., Skurdeniene I. 2010. Effect of housing system (wire cage versus group-housing) and in-house air quality parameters on the behaviour of fattening rabbits. *World Rabbit Sci. 18, 243-250. doi:10.4995/wrs.2010.777*

SAS. 2001. SAS User's Guide: Statistics. *SAS Institute Inc., Cary NC, USA.*

Schneider F., Engelhardt T., Wieser P. 2001. Characterizarion of aerosol particles from animal husbandry with single particle analytic techniques. *ASAE Meeting presentation, 1-10.*

Seedorf J., Hartung J., Schroder M., Linkert K.H., Phillips V.R., Holden M.R., Sneath R.W., Short J.L., White R.P., Pedersen S., Takai H., Johnsen J.O., Metz J.H.M., Koerkamp P.W.G.G., Uenk G.H., Wathes C.M. 1998. Concentrations and emissions of airborne endotoxins and microorganisms in livestock buildings in Northern Europe. *J. Agr. Eng. Res., 70: 97-109. doi:10.1006/jaer.1997.0281*

Sirotek K., Santos E., Benda V., Marounek M. 2003. Isolation, identification and characterization of rabbit caecal mucinolytic bacteria. *Acta Vet. Brno 72: 365-370.*

Thorne P.S., Kiekhaefer M.S., Whitten P., Donham K.J. 1992. Comparison of bioaerosol sampling methods in barns housing swine. *Appl. Environ. Microb., 58: 2543-2551.*

Watson J.G., Zhu T., Chow J.C., Engelbrecht J., Fujita E.M., Wilson W.E. 2002. Receptor modeling application framework for particle source apportionment. *Chemosphere, 49: 1093-1136. doi:10.1016/S0045-6535(02)00243-6*

Zhang Y. 2004. Indoor air quality engineering. *CRC Press, Boca Raton, Florida, 618 pp.*