# Characterization of airborne dust particles in turkey housing

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Feddes, J.J.R., Cook, H. and Zuidhof, M.J. 1992. Characterization of airborne dust particles in turkey housing. Can. Agric. Eng. 34:273-280. A single barn was sampled throughout a turkey growth cycle to investigate the size and distribution of airborne particles and to identify individual dust sources. Fecal material was found to be the main constituent of airborne dust in turkey barns. The aerodynamic properties of airborne dust and fecal material were found to be similar with an Andersen air sampler, and morphological characteristics were also similar when studied with a Scanning Electron Microscope (SEM). Results of protein analyses of fecal material and dust were also similar. Microscopically, fecal particles were found to be similar in structure to feed, but reduced in size by digestion to particles in the respirable range. Also the particle size distribution for ground fecal material and airborne barn dust were similar. Dust particle concentrations reached a maximum of 44 particles/mL in the respirable range towards the end of the growth cycle and less than 5% of the airborne particles were of non-fecal origin. The SEM was found to be superior to the Light Microscope in positively identifying particles.

Une seule grange étaient échantillonnée durant le cycle d'accroissement des dindons pour rechercher la dimension et la distribution des particules aéroportées et pour identifier les sources individuelles de poussière. Du matériel fécal étaient trouvé d'être la constitution principale des particules aéroportées dans les granges des dindons. Quand les poussières aéroportées était comparé avec la poussière du matériel fécal, les caractéristiques étaient semblable quand elles étaient étudiées avec la microscope électronique balayage (MEB), l'échantilloneur d'air Andersen et l'analyse de protéine des particules aéroportées, collectées. Les particules fécal étaient trouvées d'être dégradées à particules respirable. Aussi, la distribution de la dimension du matériel fécal broyé et des particules aéroportées étaient semblable. La concentration des particules de poussière respirables s'étende à 44 particules/mL envers le complétion de l'accroissement des dindons. Moins que 5% des particules aéroportées étaient de l'origine non-fécal. Le MEB était découvrit d'être supérieur à le microscope lumière pour identifier les particules positivement.

## INTRODUCTION

While dust is not the primary health problem in turkey barns, it can contribute to mortality by increasing susceptibility to other problems (Carpenter 1986). Dust can negatively affect rate of gain, thereby decreasing revenues of turkey operations. Poultry house aerosols consist of a wide range of particle sizes and shapes (McQuitty et al. 1985). Swine house aerosols have also been studied. They consist of solid and liquid particles ranging in size from 1 to 100  $\mu$ m (Heber and Stroik 1987). In this study, solid particles from 0.5 to 15  $\mu$ m were investigated. A proportion of these particles is in the respirable range (i.e. < 5  $\mu$ m). Since particles of this size are

not trapped by the upper respiratory tract, they are able to penetrate to the terminal air sacs of the lungs. Characterization of and distribution of dust particles from turkey facilities is of interest in order to determine the origin of particles that may have detrimental effects on turkeys.

Dust may have several effects on health and performance of livestock (Carpenter 1986), such as irritation of the respiratory tract and lowering resistance to respiratory diseases. Inhaled particles high in protein content may cause allergic reactions. Furthermore, when airborne pathogens are inspired, infection may result. High concentrations of non-pathogenic organisms may also be harmful (Carpenter 1986). Viable particles such as conidia, spores, and bacteria are generally small enough to be respirable. These particles may exist as solitary particles, or they may be attached to larger dust particles (Heber and Stroik 1987). If they reach the lungs, lesions in the terminal air sacs may result. Viable particles may be present in poultry houses in concentrations as high as 14 100 viable particles/L (Koon et al. 1963).

A respiratory disease common in turkeys is Aspergillosis. Spores of the fungus *Aspergillus fumigatus* are inhaled by the birds and create lesions which are often fatal to turkeys (Janni and Redig 1986). Fungal and bacterial infections of this sort are thought to be opportunistic, that is, they flourish when the turkey is predisposed to other health problems and, in turn, cause further weakening and possibly death in affected turkeys. In a concurrent study, five barns were sampled for aerosolized fecal material and *Aspergillus fumigatus* (Feddes et al. 1990). The authors found a stronger relationship between urates and airsacculitis than between *Aspergillus fumigatus* and airsacculitis.

Microscopic analysis of dust has been performed in swine confinement buildings (Donham et al. 1986; Heber and Stroik 1987) and in poultry houses (Koon et al. 1963). However, recent work on poultry house airborne particulate (aerosols) is scarce. Investigation of the source and composition of the particles in turkey barns is therefore necessary. With a knowledge of the type of particles that make up poultry house aerosols, the sources of the dust may be controlled or eliminated. In this project, size distribution of airborne particles in turkey housing was investigated. Also, individual sources of dust were identified and their relative contribution to the total aerosol was estimated.

## EXPERIMENTAL PROCEDURES

Dust samples were collected from a turkey barn located near

Edmonton, AB. This was done once every three weeks until the turkeys were 9 weeks of age, and then weekly until the birds were marketed. Since turkeys are most susceptible to dust related health problems during the period of 12 to 16 weeks of age, more frequent sampling was carried out towards the end of the growth cycle. A total of ten samples was obtained in a single growth cycle. Each sampling included the measurement of inside temperature and relative humidity and the collection of continuous particle size distribution for 10-40 minutes with an electronic particle sizer and an Andersen air sampler. The following data were also collected periodically: 1) bacterial counts with bacterial slit sampler for a 30 s period inside the barn, 2) potential sources of the dust in pure form (feces, feathers, feed, leg scales, beak scales, etc.), 3) dust samples collected from the fan guards (for protein analysis), and 4) dust samples taken outside the barn.

## Collection of airborne dust samples

Aerial dust samples were collected on a six-stage Andersen air sampler (Andersen 1958). The sampler was placed on the floor to collect aerosols that would likely be aspirated by the turkeys. Birds were kept from the immediate vicinity of the air sampler. All of the analyzed dust collections were made at the same location in the barn. An 11-mm and a 17-mm glass coverslip were mounted on double-sided tape on the perforated platforms underneath each stage. The 11-mm coverslip was used for scanning electron microscopy (SEM) and the 17-mm coverslip for light microscopy (LM). They were coated with a dilute solution of glue in chloroform. The chloroform evaporated, leaving a thin layer of glue onto which the airborne particles impinged during collection. Dust was usually collected for a 15 min time period at an air flow rate of 0.47 L/s. After dust sampling, one coverslip was mounted directly on an SEM stub, and the other on a glass LM slide.

## **Quantification of aerosols**

Particle size distributions were determined during each dust sample collection using a particle counter (Climet Instruments, Redlands, CA). Disturbance of the birds in the sampling area was minimized by sampling from an adjacent room via a sampling tube.

The particle counter categorized aerosol diameters into five ranges: greater than 0.5  $\mu$ m, greater than 1  $\mu$ m, greater than 2  $\mu$ m, greater than 5  $\mu$ m, and greater than 10  $\mu$ m. Each particle count was taken over a 36 s period and represented 0.28 L of air. The particle counter operated continuously over a 30-min time interval.

## **Protein analysis**

Aerosol samples were collected from the surface of exhaust fan guards inside the turkey barns. This dust was assumed to be more representative of the aerosol composition than settled dust. In settled dust, insects, fibres, and mouse feces were found to be present. These contaminants would affect the results of the protein analysis.

Two samples were obtained when the turkeys were seven weeks of age, and two more at twelve weeks of age. A duplicate protein analysis was performed on these samples using the Kjeldahl method for nitrogen determination. For comparison, samples of ground up fecal and feed material were also submitted for Kjeldahl analysis.

## **Mycological survey**

A bacteriological slit sampler was used to collect viable particles from the aerosol. The instrument was positioned at turkey level to sample ambient air similar to that inspired by the birds. Samples were collected in petri dishes containing potato dextrose agar as a growth medium. Because of its high sugar content, this medium favoured the growth of yeasts, molds, and fungi, over bacteria.

Aspergillosis is a common dust-related lung problem caused by the fungus Aspergillus fumigatus Fresnius (Chute 1984). Since it is a heat tolerant fungus, some of the agar plates were cultured at 42°C in an attempt to grow Aspergillus fumigatus selectively. The rest of the plates were incubated at 22°C. The molds were identified to genus. The following four genera were found to be present: Aspergillus, Penicillium, Cladosporium, and Mortierella.

## **Microscopic analysis**

Both a light microscope and a scanning electron microscope were used to characterize and identify the dust particles in known sources and in airborne samples. The SEM (Cambridge Stereoscan 100/250, Cambridge, England) is operated by the Department of Entomology, University of Alberta, Edmonton, and the light microscope (Bausch and Lomb, Rochester, NY) was supplied by the Department of Biology at The King's College, Edmonton. The SEM also has the capability of carrying out an elemental analysis on individual particles. The elemental analysis is done with a Tracor Northern 5500 Energy Dispersive X-Ray Analyzer (EDAX). When the sample surface is excited with the electron beam from the SEM, the resulting x-ray emission is collected and assimilated by the EDAX to determine which elements are present.

## Analysis of expected dust sources

Possible sources of aerial dust were investigated. Samples were collected and mounted on LM slides and SEM stubs for comparison with aerial dust samples. These sources included feed (barley, wheat, soybean meal, grit, and processed feed), feathers, fecal material (the uric acid coating was scraped from the feces and the two parts examined individually), bedding material (wood shavings), turkey skin, leg scales, and beak scales.

Where appropriate, the samples were dried and ground up with a mortar and pestle for approximately one minute to simulate their state in an aerosol. They were then placed in a polyethylene bottle with a perforated bottom. Air was drawn via a tube through the cap of the bottle directly to the Andersen air sampler. As air entered through the holes in the bottom of the bottle, dust became airborne and was drawn into the Andersen air sampler.

The samples that were ground up in this manner were feed, feces, and feathers. The samples broke down differently. Feed and feather samples retained their original structure, whereas fecal material did not. When drawn through the Andersen air sampler, fecal dust was transported to the lower stages of the instrument (respirable particles), whereas dust from ground feed samples was larger, less was aerosolized, and more was retained at the higher stages of the air sampler (larger particles).

## Analysis of turkey barn aerosols

Airborne particles were collected with the Andersen air sampler. The particles which were deposited on the cover slips mounted under each stage were sized on the SEM with the aid of the micron bar. On the light microscope, the particles were measured with the aid of a stage micrometer and by comparison to particles of known diameter. Size categories were chosen to represent the effect of dust on turkeys. Three categories were used:  $0-5 \,\mu m$  (respirable particles);  $5-10 \,\mu m$ (particles deposited in the upper respiratory tract); and particles greater than 10 µm. Where possible, the dust particles were identified as to their origin. This was done by comparing the SEM image of the aerosol to our collection of scanning electron micrographs of known dust sources, to documented micrographs, and by consultation (Personal Communication, G. Braybrook, Department of Entomology, University of Alberta, Edmonton, AB).

## **RESULTS AND DISCUSSION**

With the SEM analysis, fecal material was found to be the main constituent of airborne dust in turkey barns. This was confirmed by the fact that both the main portion of turkey barn aerosol and pulverized fecal material were transported to the lower two levels of the Andersen air sampler (corresponding to the respirable range). Upon grinding, fecal material was the only dust source that broke down into particles that were fine enough to be transported to the lower levels of the Andersen air sampler.

## Fecal material analysis

*Urates.* Consistent round spheres of material (Fig. 1) were interspersed with digested feed particles. They were similar to starch granules, but were smaller and less angular. They ranged in size from 1 to 12  $\mu$ m in diameter with the majority of them in the 3 to 8  $\mu$ m range. By separating the white uric acid coating from the feces, and examining them separately under the SEM, the source of these "fecal spheres" was determined. The white coating of the feces contained only these spheres. Therefore, it was concluded that the spheres were crystals of uric acid, the nitrogenous waste product of birds.

It is important not to confuse these uric acid crystals with small starch particles. Typical starch grains in wheat, barley, and soybean meal range in size from 2 to 25  $\mu$ m in diameter with the majority between 12 to 17  $\mu$ m. Starch grains are more angular. Starch grains also typically expand and crack the gold coating if exposed to an intense electron beam on the SEM. Uric acid crystals do not expand in this way.

Digested feed. Feed which has undergone the digestion process has some distinct characteristics. Although some of the particles are difficult to distinguish from undigested feed, the majority can be identified with the SEM. The digestion process of turkeys appears to effectively remove starch from the feed. Fecal particles resemble feed particles, but are usually less irregular in shape and have rounded edges (Fig. 2) as opposed to the sharp morphology of feed (Fig. 3). Fecal particles range in size from approximately 1 to 40 min aerosols, with the majority falling in the 3 to 7  $\mu$ m range. Particles are more distinguishable with the SEM than with the LM.



Fig. 1. Scanning electron micrograph - Urates in turkey feces. 1100X.

## Characterization of dust particles - Light microscope

The results presented in Table I list the numbers of particles collected from all six stages of the Andersen air sampler. These data represent a sum of particles counted from four aerosol collections at various stages throughout the turkey growth cycle (6, 8, 9, and 14 weeks of age). Qualitatively, there were some minor differences in dust characterization at different times during the growth cycle. Fecal and feed categories were grouped together in the light microscope analysis because these particles are difficult to differentiate. A number of representative fields were chosen in which every particle was counted.

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Fig. 2. Scanning electron micrograph - Fecal particle (note smooth eroded appearance). 5410X.



Fig. 3. Scanning electron micrograph - Feed particle from turkey ration (note: irregular appearance, angularity of the starch grains). 1400X

## Characterization of dust particles - Scanning electron microscope

Particles were counted in a similar fashion with the SEM. All six stages were analyzed from each collection. Fields in which particles were to be counted were chosen randomly by moving the stub and zooming in on a field. Randomizing field selection on the SEM was easier than on the LM due to the ease of zooming into a field.

The results of SEM analysis suggest that fecal material comprised a significant proportion of the dust, especially in the respirable range. A typical aerosol sample is shown in Fig. 4. The smaller particles are urates and fecal particles. These are difficult to distinguish at low magnification (340X), whereas at higher magnifications (750X) the spheres can be easily identified. At high magnifications, fecal particles can be identified by their lost structural integrity (tattered, thin, pointed, eroded, small fragments), and specks of material can often be seen on the particles (dirty appearance). The feed particles in Fig. 3 are larger and cleaner in appearance and have noneroded surfaces. Also, several starch particles are present. Starch particles readily expand and crack the gold coating under the intensity of the electron beam. Table II presents the total number of particles counted for the three sets of samples collected with the Andersen air sampler at 9, 11, and 13 weeks of age.

The results of an analysis of a single dust sample collected outside of the barn with the Andersen air sampler are presented in Table III. The collection was taken at ground level outside the barn adjacent to inside where the dust samples were taken. This was done to determine the degree to which the aerosol that entered



Fig. 4. Scanning electron micrograph - Typical aerosol sample (smaller particles are urates and fecal particles). 340X

# Table I. Particle numbers and sizes of a randomsubsample of barn aerosol particles asidentified with the Light Microscope.

Particle type	0-5 µm	5-10 μm	>10 µm
Urates (including aggregations)	207	294	104
Fecal/feed	1577	587	360
Fecal-feed (urates attached)	1	11	79
Starch	8	28	19
Feather		2	51
Scales		2	76
Skin			1
Conidia	1	3	
Trichomes (plant hairs)			13
Wood			2
Glass	6	12	9
Other:			
Long and thin	3	11	10
Angular	100	31	17
Fibrous	6		7
Round	16	1	
Mealy (complex rough texture)		4	24
Total	1925	986	772

the barn was responsible for the dust inside. Particles from all six stages of the Andersen air sampler were counted. The complete range of particle sizes is therefore represented.

The Energy x-ray dispersion analysis of SEM samples indicated that particles of fecal origin had higher potassium (K) content than those of nonfecal origin (Fig. 5). The feed sample spectra showed a low K content. This x-ray elemental analysis confirmed the presence of fecal material in the airborne dust.

In pig housing studies, Heber and Stroik (1987) and Donham et al. (1986) found that a high proportion of the airborne particles was starch. To make a comparison, several samples of pig barn aerosols were obtained from two pig barns near Ponoka, AB, and one at the Edmonton Research Station, University of Alberta, Edmonton. Airborne particles in these samples contained more starch than those collected from turkey barns. An SEM analysis indicated

that pig feces contain significant quantities of starch. Ground pig feces, like turkey feces, also break down into smaller particles more readily than feed particles. This suggests that fecal material is more readily aerosolized and that it may be the primary source of dust in pig barns.

## **Concentration of airborne particles**

Particle counts were placed into two size categories, namely, greater than 5  $\mu$ m and less than 5  $\mu$ m. These values were obtained over an 0.5h period during mid-day when activity levels were moderate. During the early stages of the growth cycle, particle counts ( $<5 \mu m$ ) were high. This may be due to shedding of down as observed in broilers (Feddes et al. 1984) and dryness of the litter. As the birds matured, the litter became moist, and the turkeys were less active. Counts between 8 and 14 weeks ranged between 16 and 44 particles/mL (Table IV). This compares with a maximum daily concentration of 6 particles/mL for broilers (Feddes et al. 1984), 13 particles/mL for laying hens (McQuitty et al. 1985), 27 particles/mL for broiler breeders (O'Connor et al. 1988), and 100 particles/mL in pullet rearing facilities (Glennon et al. 1989). The particle concentrations presented in Table IV demonstrate that dust levels in the respirable range are high. Turkeys that are exposed to these dust levels over a growth period may be expected to suffer ill effects from the dust. The mean temperatures and relative humidities did not appear to be related to the change in particle concentration (Table IV).

## **Protein analysis**

In the Kjeldahl nitrogen determination, only the amine and amide (organic) nitrogen of a sample is measured, while the nitrate (inorganic) nitrogen is removed as nitric acid. Equivalent protein content (EPC) is calculated by multiplying the nitrogen content by 6.25. Uric acid, the nitrogenous waste product of turkeys, contains 33% nitrogen (206% EPC). The



(a) Feed particle



(b) Fecal particle



(c) Typical airborne fecal particles

## Fig. 5. Energy X-ray dispersive analysis of SEM samples showing typically higher potassium content in fecal particles (b and c) and lower content in feed (a).

# Table II. Particle numbers and sizes of particles from a<br/>random subsample of barn aerosol as<br/>identified with the Scanning Electron<br/>Microscope.

Aerosol type	0-5 µm	5-10 µm	>10 µm
Urates	233	48	
Aggregations of urates	59	51	13
Fecal	736	351	249
Feed	1	11	32
Feed/fecal	8	6	2
Starch		5	6
Feather dandruff	1	4	41
Feather spine (length)			27
Trichomes		1	3
Pollen			1
Dirt (mineral)	4		8
Scales		4	166
Spores	3		2
Plant parts	1	7	3
Insect parts			1
Skin			1
Barley beards			1
Bran			3
Glass	4	12	17
Other:			
Grainy		2	1
Round	1		
Platy		2	2
Total	1051	504	581

results of the Kjeldahl analysis indicate a very high level of organic nitrogen in the aerosols (Table V). The two samples collected when the turkeys were 7 and 12 weeks of age averaged 12.1% N and 12.2% N, respectively. Uric acid was approximately 2% of the total feces while counts indicated that 30% of the airborne fecal particles were uric acid spheres (Table II). Evidence from both microscopic analysis and protein analysis suggests that uric acid crystals comprise a large portion of turkey house aerosols.

## **Mycological analysis**

When the turkeys were 9 to 14 weeks of age, three agar plates were exposed to both inside and outside air on a weekly basis. Data on the colony forming particles (CFPs), which were incubated at 22°C, are presented in Table VI. For one of the sampling times (12 weeks of age) the CFP count was higher outside than inside. Initially, the outside CFP counts were assumed to be low. Consequently, air was sampled over a longer period of time to increase exposure time of the plates to the sample air. Overcrowding of CFP's was evident in the sample taken at 12 weeks.

Some of the agar dishes were incubated at a higher temperature to determine whether or not Aspergillus fumigatus was present. The results of this test showed that there were

Aerosol type	0-5 μm	5-10 µm	>10 µm
Aggregations of urates		3	
Fecal	16	18	33
Feed		5	15
Feed/fecal	2	2	
Feather dandruff			2
Feather spine (length)			3
Trichomes			2
Soil (mineral)	29	40	36
Sand	2	5	2
Clay			1
Metal fragment			1
Glass		1	2
Scales			3
Spores	2	25	3
Plant parts		2	20
Insect parts	1		1
Skin			1
Total	52	101	125

Table III. Particle numbers and sizes of aerosol particles collected outside the building as identified with the SEM\*.

\*25-minute sample time - random subsample

Table IV. Summary of environmental data and airborne particle concentrations\*

Age	Temperature	Relative Humidity	Particl	es/mL
	°C	%	<5 µm	>5 µm
5	17	40	81	8
7	17	70	91	15
8	22	77	16	2
10	31	52	16	4
11	25	71	24	5
12	23	65	44	11
13	29	54	34	5
14	26	52	35	6

\* data obtained over 0.5 hour period near mid-day.

heat tolerant fungi (*Mortierella* spp.), but none of the CFPs were *Aspergillus* species. In the indoor samples from week 14, the six colonies that grew were all *Mortierella* species. Similarly, the samples from week 9 that were incubated at 42°C showed positive results for only a single *Mortierella* colony in each case. There were no *Aspergillus* colonies evident in any of the heat-tolerance tests.

## SUMMARY AND CONCLUSIONS

Fecal material was the main constituent of airborne dust in the turkey barn in this study. It contained both digested feed particles and uric acid crystals. The SEM was better able to

<b>Fable V.</b>	Nitrogen and equivalent protein content of	)f
	aerosols, uric acid, feed and feces.	

Sample	% Nitrogen	% Equiv. Protein
aerosol: 7wks	12.1	75.6
aerosol: 7wks	12.0	75.1
aerosol: 12wks	11.9	74.1
aerosol: 12wks	12.6	78.7
uric acid	33.0	206.0
feed	2.6	16.0
feces (6 samples)	6.5	40.6

Table VI. Colony Forming Farticles (CFFS) at 22	fable VI.	VI. Colony	<sup>7</sup> Forming	Particles	(CFPs)	at 22°	C
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Bird Age (wks)	Inside CFP/L	Outside CFP/L
9	4.4	3.8
10	4.9	3.4
11	5.7	1.7
12	2.3	2.6
14	1.5	0.8
Average	3.8	2.5

differentiate between feed and fecal particles than the LM, although both types of microscope showed that the majority of the particles were in the respirable range. Concentrations of dust particles exceeded those previously found in broiler housing, broiler breeding, and laying hen houses. Only dust levels in pullet rearing facilities exceeded those found in turkey housing. The mycological analysis indicated that viable colony forming particles existed both outside and inside the turkey facilities in a ratio of approximately 1:2, respectively.

The following conclusions can be drawn from this experiment:

1) The SEM is superior to the LM in identifying particles since it provides a better 3-D image of particles, and since it shows surface characteristics of the particles.

2) Airborne particles in turkey housing are primarily comprised of fecal material. The high nitrogen content, identification of the source in the airborne dust samples, and fecal material reducing to particles in the respirable range upon grinding with a mortar and pestle confirmed this.

3) The particle size distributions for pulverized fecal material and airborne dust in a poultry environment were similar.

4) Dust particle concentrations reached a maximum of 44 particles/mL in the respirable range late in the growth cycle.

## ACKNOWLEDGEMENTS

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