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Adverse and Benefic Effects of Airborne Endotoxins on Human and Poultry Health – Monographic Study

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Abstract. Endotoxins are powerful pro-inflammatory agents, producing systemic effects and lung obstruction, even at low level of exposure. It is well known that farmers had a major risk to develop professional respiratory diseases. It was proven that exposure to organic dust could be substantial and can lead to respiratory diseases and to an annual accentuated deprivation of lung functions, endotoxins representing thus a significant respiratory risk. Exposure to airborne endotoxins is associated with a variety of acute (cough, fever, malaise, temporary decrease of lung function) and chronic respiratory symptoms, for example the toxic pneumonia and chronic obstructive bronchitis. It was considered that broilers housed on permanent bedding should present a desensitized immune system, which should demonstrate a better tolerance or resistance to endotoxins exposure, compared to caged broilers.

Key words: airborne endotoxins, poultry, human, asthma, inflammatory process

ADVERSE EFFECTS OF ENDOTOXINS ON PEOPLE HEALTH

In 1974, Thomas, cited by Douwes *et al.* (2003) suggested that "endotoxins are read by our tissues as the very worst of bad news" and "in response to these molecules we are likely to turn on every defence at our disposal". This characterization tremendously underlines the toxic potential of these macromolecules.

Endotoxins are powerful proinflammatory agents, producing systemic effects and lung obstruction, even at low level of exposure (Casey *et al.*, 2006, Michel *et al.*, 1997, Thorne, 2002). They are considered pyrogen, because they induce fever.

It is well known that farmers had a major risk to develop professional respiratory diseases. An European study demonstrate that those who present the biggest risk of developing respiratory symptoms due to their activity are the swine breeders from Denmark and Germany and poultry breeders from Switzerland (Radon *et* al, 2002).

Although numerous studies presents methodological deficiencies, although there is a heterogeneity of sampling times, of determination and quantification techniques, of diagnosis criteria, it was proven that exposure to organic dust could be substantial and can lead to respiratory diseases and to an annual accentuated deprivation of lung functions, endotoxins representing thus a significant respiratory risk (Omland, 2002). The establishment of a dose – response relation is problematic, because there are no standard procedures for sampling and analyzing (Reynolds *et al.*, 2002). The concentration of endotoxins from total suspended particles (TSP) is negatively correlated to FVC (*Forced Expiratory Vital Capacity* – the volume change of the lung between a full inspiration to total lung capacity and a maximal expiration to residual volume) and FEV_1 (*Forced Expiratory Volume in 1 second* – the

volume exhaled during the first second of a forced expiratory maneuver started from the level of total lung capacity) (Weber, 2001).

The LPS complex represents the major antigen of Gram negative bacteria, recognized by the immune system of superior organisms, many aspects of tissue destruction caused by infections with Gram negative bacteria could be reproduced, mimed through LPS administration. The biological effects of LPS were intensively studied on mammals. LPS are capable of stimulating many immunocompetent cells and activation of cytokines, leading to release of pro-inflammatory mediators (IL1, IL-6, IL-8 and TNF-alpha), which initiates the response that culminates with fever, headache, malaise, respiratory symptoms (dry cough, tight chest feeling) and decreases in lung function, metabolic alterations and changes in vascular permeability and resistance. Meanwhile, multiple control mechanisms, including anti-inflammatory cytokines, are activated, they can limit and eventually end the inflammatory response (Wang *et al.*, 2003).

The balance of pro- and anti-inflammatory cytokines determines the net response of the host. It is generally accepted that the overproduction of pro-inflammatory cytokines in severe sepsis causes damage to the endothelial cells, changes in vascular tonus, disseminated intravascular coagulation (DIC) and other complications that ultimately lead to multiple organ loss and death (Bruce, 2002, Wang *et al.*, 1997).

In literature, data about absorption and distribution of endotoxin after inhalation could be rarely found. Endotoxin particles deposited in upper airways are cleared by mucociliary transport, and those that reach deep into the airways is assumed to be eliminated by macrophages and polymorphonuclear leukocyte phagocytosis (Heederik and Douwes, 1997).

Numerous studies, experimental and epidemiological, documented adverse respiratory effects due to inhalation of relatively low endotoxin concentrations, around the standard threshold, respectively 5 to 10 ng/m^3 (50-100 EU/m^3) (McNeel and Kreutzer, 1997, Wouters *et al.*, 2006).

Acute airways obstructions occur at concentrations between 45 and 330 EU/ air m^3.

The amount of allergen necessary to produce a certain air expiration ability reduction was much lower in subjects initially exposed to endotoxin (Boehlecke *et al.*, 2003). Sensitive individuals exhibit symptoms at endotoxin levels of 6,5 ng or even lower, while healthy individuals show no symptoms of bronchial disorders, even if they are exposed to six times higher levels.

Reed and Milton, cited by Bruce (2002), launched the hypothesis that airborne endotoxins adversely affect patients with asthma, by increasing the severity of airways inflammation, and by increasing the susceptibility to rhinoviruses induced (caused) colds. It was demonstrated an additive or synergistic effect of inhaled endotoxins with asthma responsible allergens. Bacterial endotoxins enhance the inflammatory response to inhaled allergens, endotoxin exposure is considered a possible factor in increasing the frequency of asthma worldwide (Boehlecke *et al.*, 2003).

Researchers at the University of Iowa found that women are generally more sensitive to endotoxin than men, but there are susceptible individuals regardless of gender.

Inhalation of certain doses of LPS produces both pure bronchial inflammatory response and a systemic response. Information about dose - response relation to inhaled LPS in normal subjects is the prerogative to define exposure thresholds (Michel *et al.*, 1997, Radon *et al.*, 2002).

A study conducted on nine healthy subjects, exposed through inhalation to different doses of endotoxins (blind, 0,5 µg, 5 µg and 50 µg of *E. coli* LPS) reveals that the most sensitive markers of inflammation induced by LPS are represented by the change in the number of polymorphonuclear cells (PMN) and their activation level, the blood level of C reactive protein concentration, but also

the number of PMN in sputum. The limit value for acute inhalation of LPS, a value that does not produce any response from the body is less than 0.5 μg, corresponding to an exposure within 10 hours, less than 50 ng airborne LPS / air m^3 (Michel *et al.*, 1997).

Acute effects generated by endotoxins

By inhalation of aerosols containing endotoxin derived from E. coli, in doses of 2, 10 and 20 μg, it was demonstrated that acute effects that occur are dry cough and shortness of breath, accompanied by a decrease in FEV_1. Inhalation of endotoxins from other bacterial genera has led to similar symptoms, accompanied by a slight fever and malaise. Numerous studies have demonstrated strong reactions of airways on asthmatic people with bronchial hyper- reactivity, after experimental exposure. LPS-induced bronchial obstruction was associated with specific non-immune inflammatory processes, rather than inflammation induced by IgE.

An effective concentration, after which 50% of those exposed show a decrease of 5% or more in FEV_1, was calculated as equal to 100 ng/m^3, for a 10% decrease in FEV_1 the dose required (suggested) is 10 times higher (1μg / air m^3) (Douwes *et al.*, 2003).

Many of the experimental or epidemiological studies support the hypothesis that inhaled endotoxins have a causative role in acute lung response, dose-dependent. However, cannot be ruled out participation of other components of organic dust in the development of acute pulmonary effects (Douwes *et al.*, 2003).

Recent experiments have suggested that the combined presence of glucans and bacterial endotoxin lead to a more severe airway inflammation, compared with the inflammation obtained by individual actions, and this combination of bacterial and fungal chemical can cause long-term changes in lungs (McNeel and Kreutzer. 1997).

Repeated exposures over a period of five weeks to endotoxin aerosols, to β-1-3-glucan aerosols or to a combination of these substances, lead to an increased number of inflammatory cells in the airways of exposed animals. Combined exposure leads to a further increase in the number of cells, an increase of alveolar infiltrates and to an early appearance of granuloma (Douwes *et al.*, 2003).

Chronic effects generated by endotoxins

Epidemiological studies support that chronic respiratory dysfunctions are correlated with endotoxin doses.

A major finding of these studies is the chronic injury of lung function, more obviously related to exposure to endotoxin than to dust exposure, thus suggesting the role of endotoxin in the etiology of chronic occupational lung diseases. Interesting is that these studies show that chronic deterioration in lung function is correlated with exposure to endotoxin at the same levels described for acute experimental studies. Exposure limits suggested in some studies are less than 20 ng / m^3, which is in remarkable agreement with experimental results mentioned above, respectively 9 ng / air m^3 air (Douwes *et al.*, 2003).

Mechanism of action: endotoxins are capable of inducing a wide range of inflammatory reactions both in vivo and in vitro. Shortly, the body response when affected by endotoxin is mediated by endogenous cytokines and metabolites (IL-1, IL-6, IL-8, TNF-α, arachidonic acid) that are produced by various cells in the airways such as alveolar macrophages, vascular and epithelial cells, polymorphonuclear cells. Macrophages and monocytes carry specific receptors for LPS binding (CD14, which plays a crucial role in

activating these cells and subsequent inflammatory reactions). The LPS binding to CD14 receptors is catalyzed by a LPS-binding protein (LBP). There are also components that prevent LPS binding to receptors on target cells, by neutralizing LPS. One of the most important is a bacterial protein which increases the permeability, called bactericidal permeability increasing protein – BPI. BPI is competing with LBP to bind LPS and subsequent binding to CD14, acting as a feedback inhibitor that prevents in the infected hosts the limitation of septic reactions. These endotoxins induce inflammatory responses in the airways, generating acute or chronic obstruction, which is manifested by obstructive respiratory symptoms and lung function decrease (Chahoud *et al.*, 2009, Douwes *et al.*, 2003).

Respiratory symptoms due to exposure to organic dust can range from mild acute condition that, at first, hardly affects daily life, to severe chronic respiratory diseases, that require special care. Weak acute changes in lung function and mild respiratory symptoms are predictive of chronic respiratory diseases, so that should be taken seriously into account.

Chronic obstructive respiratory diseases are characterized by airway obstruction and subsequent limitations of the volume of expired air, plus the presence of respiratory symptoms. Chronic restrictive airway diseases (fibrosis, hypersensitivity pneumonia) may also occur (in combination with or separately from airway obstruction), but are much less frequent in workers with dust exposure.

Most serious obstructive states can be grouped into two broad categories: chronic obstructive pulmonary disease (COPD, including chronic bronchitis and emphysema) and asthma. Airway obstruction in COPD is primarily irreversible, while airway obstruction in asthma is mainly reversible.

It is important to note that pre-existing respiratory status and other host factors (atopy, smoking) may change the risk of respiratory symptoms related to work. For example, workers who have asthma because of exposure to house mites dust may experience exacerbations of asthma due to exposure to organic dust at levels that will not induce any symptoms on healthy people (Douwes *et al.*, 2003).

Literature suggests an association between exposure to bacteria or bacterial endotoxins in confined spaces and various adverse respiratory healths, including:

- Intensified, exacerbated asthma;
- increased incidence of respiratory symptoms, including cough and dyspnoea;
- increased incidence of upper airway infections;
- chronic bronchitis and emphysema;
- inflammation of the airways;
- hypersensitivity pneumonia (Bruce, 2002).

A lot is already known about exposure to dust containing microorganisms and endotoxin in different working conditions and the relationship to respiratory symptoms and disease and the interest was focused on work in swine confinement buildings because the highest exposure and the highest frequency of symptoms is found here. Poultry farming also carries a substantial (if not higher than swine farming) exposure to dust, but the number of working hours spent inside and the number of persons employed is much lower than in swine farming (Radon *et al.*, 2002).

Socio-economic changes with lead to disappearance of small family-based farms and the development of agricultural industry in pig and poultry farming with all working hours spent inside the confinement building has increased exposure and will continue to do so in the future and will have a major impact on the respiratory health of the agricultural workers. Numerous researches

emphasize that working inside the building double or triple the incidence of respiratory symptoms and there is a clear dose - response relation, according to the number of hours of exposure.

Radon *et al.* (2002) affirm that the problem is mostly represented by irritation of the airways with other possible disease mechanisms (allergic alveolitis, allergic asthma, Organic Dust Toxic Syndrome, emphysema, lung fibrosis) being either rare or of no significance.

Noteworthy, there is a discrepancy between results from acute exposure studies and results from long term studies. Probably some of the explanation of this is the use of different populations of persons, where some are selected after years of exposure in farming and others, mainly the persons used in acute experiments, are so called naive subjects to this environment.

The exposure threshold establishment in animal confinement buildings is very important, in order to avoid respiratory disease on next generation farmers (Radon *et al.*, 2002).

ADVERSE EFFECTS OF ENDOTOXINS ON POULTRY HEALTH

Bakutis *et al.* (2004) state that epidemiological and experimental studies provided sufficient data in order to identify criteria for professional influence, but very little is known about endotoxin effect upon sheltered animals.

Housing conditions determine the alteration of sanguine leucocytes profile, one hypothesis state that broilers housed on permanent bedding should present a desensitized immune system, which should demonstrate a better tolerance or resistance to a subsequent LPS intravenous exposure, compared to caged broilers (Wang *et al.*, 2003).

Experimentally, intravenous injection of *Salmonella typhimurium* LPS induced rapid inflammatory responses in boilers as indicated by leukopenia, lymphopenia, heteropenia, monopenia, basopenia, and thrombocytosis at one hour post-injection (Wang *et al.*, 2003).

Prior exposure to LPS could induce a state of immune system paralysis known as a LPS tolerance. This immune system paralyses could be an adaptive response, whose aim is to protect the host from inflammatory injuries. Thus, responsiveness to LPS may differ in broilers, according to house system (permanent bedding or cages) (Wang *et al.*, 2003).

BENEFIC EFFECTS OF ENDOTOXINS ON PEOPLE HEALTH

Exposure to certain levels of endotoxin is probably essential for normal development of human and animal immune system (Burge and Gallup, 2005). Endotoxins induce the systemic and local inflammation, also present some anti-tumor effects by activating macrophages to produce alpha tumor necrosis factor and interferon, thus reducing the risk of cancer and increasing the effectiveness of vaccines. Numerous studies have shown that groups of people exposed to high concentrations of airborne endotoxins (farmers) have low rates of lung cancer (Lange *et al.*, 2003, McNeel and Kreutzer, 1997, Rylander, 2002).

Perhaps the level of exposure to endotoxins and other bacterial wall components represents a protective factor in the development of atopic diseases during childhood (Rylander, 2002, Von Mutius *et al.*, 2000).

According to one hypothesis, the chronic inhalation of endotoxin by man can increase nonspecific immune response to antigens (adjuvant effect) through activation of B cells. However, currently there is no direct evidence to support this hypothesis. Also, no data showing carcinogenic, mutagenic or reproductive effects from exposure to endotoxin are available (Heederik and Douwes, 1997, McNeel and Kreutzer, 1997).

Recent researches suggested that exposure to airborne bacterial endotoxins in childhood, by growth in the agricultural environment, may reduce further development of atopia, asthma and

allergic responses, by generating a strong cytokines response and tolerance to allergens. This mechanism was postulated by Reed and Milton to explain a reduced incidence of asthma in children who grew up in agricultural environment than those raised in urban areas. In residential environments, exposure to endotoxin appears to be related to an increased risk of asthma. This effect is called the hygiene hypothesis, which considers that lowering the exposure to endotoxin due to increased hygiene standards led, in part, to increased incidence of asthma (Bruce, 2002, Burge and Gallup, 2005, Omland, 2002, Park *et al.*, 2001).

BENEFIC EFFECTS OF ENDOTOXINS ON POULTRY HEALTH

Depending on quantity and the administration route, LPS are capable to produce benefic effects on host. For example, through infection, small quantities of LPS gain access to body fluids and organs, and intestinal translocation is benefic for the host by the stimulation of the immune system, which will lead to a increased resistance to infections. It was demonstrated that broilers exposed to *E. coli* LPS show better antiviral activity against Newcastle disease virus (Wang *et al.*, 2003).

The available literature suggest that birds and mammals respond in a similar manner to LPS, by production of cytokines and antibodies and similar hemodynamic modifications, however LPS tolerance is higher in birds than in mammals. However, there is relatively little information on the systemic response of broilers to LPS (Wang *et al.*, 2003).

CONCLUSIONS

- Exposure to airborne endotoxins is associated with a variety of acute (cough, fever, malaise, temporary decrease of lung function) and chronic respiratory symptoms, for example the toxic pneumonia and chronic obstructive bronchitis.
- Epidemiological and experimental studies provided sufficient data in order to identify criteria for professional influence, but very little is known about endotoxin effects upon sheltered animals, especially on poultry.

REFERENCES

1. Bakutis B., E. Monstviliene and G. Januskeviciene (2004) Analyses of Airborne Contamination with Bacteria, Endotoxins and Dust in Livestock Barns and Poultry Houses. Acta Vet. Brno 73:283–289.
2. Boehlecke B., M. Hazucha, N.E. Alexis, R. Jacobs, P. Reist, P.A. Bromberg and D.B. Peden (2003) Low-dose airborne endotoxin exposure enhances bronchial responsiveness to inhaled allergen in atopic asthmatics. J Allergy Clin immunol. 112(6):1241–1243.
3. Bruce S. M. (2002) Indoor Air Pollutants In Residential Settings - Respiratory Health Effects and Remedial Measures to Minimize Exposure, A Review for The Ontario Lung Association, P. Eng. Small and Fleming Limited
4. Burge H. and D. Gallup (2005) Endotoxins. The Environmental Reporter, EMLab, 3(3).
5. Casey K.D., J.R. Bicudo, D.R. Schmidt, A.Singh, S.W. Gay, R.S. Gates, L.D. Jacobson and S.J. Hoff (2006) Air Quality And Emissions From Livestock And Poultry Production/Waste Management Systems – Animal Agriculture and the Environment: National Center for Manure and Animal Waste Management White Papers. St. Joseph, Michigan, ASABE, 1-40.
6. Chahoud, M., B. Zucker and U. Roesler (2009) - Characterisation of the endotoxic and proinflammatory activity of different airborne dust fractions from animal houses. Proceedings of the XIV ISAH Congress, Vechta, Germany, 2:913–916.

7. Douwes J., P. Thorne, N. Pearce and D. Heederik (2003) Bio aerosols: health effects and exposure assessment. Ann Occup Hyg 47:187-200.
8. Heederik D and J. Douwes (1997) Towards an occupational exposure limit for endotoxins?. Ann. Agric. Environ. Med. 4:17–19.
9. Lange J.H., G. Mastrangelo, U. Fedeli, R. Rylander and D.C. Christiani (2003) There is an alternative reason for lower-than-expected rates of lung cancer in farmers. Arch Environ Health 58(5):316-317.
10. McNeel S. and R. Kreutzer (1997) Bioaerosols And Green-Waste Composting In California. Final Report Printed by California Department of Health Services Environmental Health Investigations Branch Oakland, California.
11. Michel O., A.M. Nagy, M. Schroeven, J. Duchateau, J. Nève, P. Fondu and R. Sergysels (1997) Dose-Response Relationship to Inhaled Endotoxin in Normal Subjects. Am. J. Respir. Crit. Care Med. 156(4):1157–1164.
12. Omland O. (2002) Exposure and respiratory health in farming in temperate zones-a review of the literature. Ann. Agric. Environ. Med. 9(2):119-136.
13. Park J.H., L.D. Spiegelman, R.D. Gold, A.H. Burge and D.K. Milton (2001) Predictors of Airborne Endotoxin in the Home. Environmental Health Perspectives 109(8):859-864.
14. Radon K., E. Monso, C. Weber, B. Danuser, M. Iversen, U. Opravil, K. Donham, J. Hartung, S. Pedersen, S. Garz, D. Blainey, U. Rabe and D. Nowak (2002) Prevalence And Risk Factors For Airway Diseases In Farmers - Summary Of Results Of The European Farmers' Project. Ann. Agric. Environ. Med. 9:207–213.
15. Reynolds S.J., P.S. Thorne, K.J. Donham, E.A. Croteau, K.M. Kelly, D. Lewis, M. Whitmer, D.J. Heederik, J. Douwes, I. Connaughton, S. Koch, P. Malmberg, B.M. Larsson and D.K. Milton (2002) Comparison of endotoxin assays using agricultural dusts. AIHA J (Fairfax, Va) 63(4):430-438.
16. Rylander R. (2002) Endotoxin in the environment – exposure and effects. Journal of Endotoxin Research 8(12):241-252.
17. Thorne P.S. (2002) Air Quality Issues, p.35-47. In: Iowa Concentrated Animal Feeding Operations Air Quality Study. ISU/UI Study Group, University of Iowa College of Public Health, Iowa City, IA.
18. Von Mutius E., C. Braun-Fahrländer, R. Schierl, J. Riedler, S. Ehlermann, S. Maisch, M. Waser and D. Nowak (2000) Exposure to endotoxin or other bacterial components might protect against the development of atopy. Clinical & Experimental Allergy 30(9):1230-1234.
19. Wang W., R.F. Jr. Wideman, M.E. Chapman, T.K. Bersi and G.F. Erf (2003) Effect of intravenous endotoxin on blood cell profiles of broilers housed in cages and floor litter environments. Poult. Sci. 82(12):1886-1897.
20. Wang Z., K. Larsson, L. Palmberg, P. Malmberg, P. Larsson and L. Larsson L (1997) Inhalation of swine dust induces cytokine release in the upper and lower airways. Eur. Respir. J. 10:381–387.
21. Weber C. (2001) Obstructive Airway Diseases In Swiss Farmers. The Swiss Federal Institute Of Technology, PhD Diss. ETH No. 14164, Abstr. 1-8
22. Wouters, I. M., S. Spaan, J. Douwes, G. Doekes and D. Heederik (2006) Overview of personal occupational exposure levels to inhalable dust, endotoxin, beta(1-->3)-glucan and fungal extracellular polysaccharides in the waste management chain. The Annals of occupational hygiene 50(1):39-53