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LITTER AERATION DURING THE REARING CYCLE OF BROILERS: PRODUCTIVE AND WELFARE IMPLICATIONS

Tesis de Máster

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ABSTRACT

Litter quality is one of the main parameters in poultry production. High moisture content of litter may lead to poor welfare conditions by increasing lesions and reducing broiler productive performance. Litter aeration arises as an alternative to reduce litter moisture during the productive cycle of broiler chickens. The objective of this study was to evaluate litter aeration during a broiler rearing cycle, from the perspective of health, welfare and performance of the animals. For this purpose, an experimental broiler farm with three identical rooms was used in the study. Two rooms were assigned to the litter aeration treatment, while the other one served as control room. During the rearing cycle, physical and chemical properties of bedding material were analysed. Environmental concentrations of ammonia and particulate matter were also measured. In addition, production parameters as well as the condition of the pads, breast, hocks and conjunctive lesions were assessed. In addition, at the end of the cycle, other indicators of welfare such as tonic immobility and injuries in the respiratory tract, heart weight, status of the right atrium, hidropericardium, ascites, corneal ulcer and tibial dyschondroplasia were also evaluated. The results showed a reduced effect of litter aeration on bedding material properties. Environmental ammonia concentrations were higher for the group where litter aeration was performed but below the threshold that has been proven to be harmful for animals. However, concentrations of particulate matter in the air exceeded the limits recommended for human and animal health in both groups. Finally, as regards animals, chickens subjected to litter aeration showed poorer growth rate and, worse feed conversion rate than animals from control rooms. There were no relevant differences in the number of chickens with lesions in the skin of breasts, hocks and pads. No significant differences in the prevalence of conjunctivitis were found either. Regarding animal health indicators, there was observed only a higher prevalence of tibial dyschondroplasia in broilers subjected to litter aeration, although lesions were mostly mild.

RESUMEN

La calidad de la cama es uno de los principales parámetros en la producción de pollo de engorde. Altos niveles de humedad en la cama, pueden empeorar las condiciones de bienestar animal, incrementando algunas lesiones y reduciendo la productividad de los pollos. El volteo de la cama surge como una alternativa para reducir la humedad de la cama durante el ciclo productivo de los pollos de engorde. El objetivo de este estudio fue evaluar el efecto del volteo de la cama durante un ciclo productivo de pollos de engorde desde el punto de vista de la salud, bienestar y productividad de los animales. Para ello, se utilizó una granja experimental con tres salas idénticas, de las cuales dos se destinaron para el tratamiento mientras que la otra se destinó como sala control. Durante el ciclo productivo se analizaron las propiedades físico-químicas del material de cama. También se midieron las concentraciones ambientales de amoniaco y material particulado. Además, se valoraron los parámetros productivos y el estado de las almohadillas, pechugas, corvejones y lesiones conjuntivas de los animales. Asimismo, al final del ciclo se valoraron otros indicadores de bienestar como inmovilidad tónica, lesiones en el aparato respiratorio, peso del corazón, estado de la aurícula derecha, hidropericardio, ascitis, úlcera corneal y discondroplasia tibial. Los resultados mostraron un efecto reducido del volteo sobre las propiedades del material de la cama. Las concentraciones ambientales de amoniaco resultaron ser más altas para los animales del grupo sometido al volteo de la cama pero en todo momento estuvieron por debajo del umbral que se ha demostrado nocivo para los animales. Sin embargo, las concentraciones de material particulado en el aire sí que superaron los límites recomendables para la salud humana y animal en ambos grupos. Finalmente, en relación con los animales, las salas sometidas al tratamiento presentaron un consumo de alimento similar pero inferior crecimiento y, consecuentemente, un peor índice de conversión. No se observaron diferencias relevantes en el número de animales con lesiones en pechugas, corvejones, almohadillas, ni en los afectados por conjuntivitis. En relación con los indicadores de salud de los animales, solamente se observó una mayor incidencia de discondroplasia tibial en los pollos sometidos al volteo de la cama aunque las lesiones fueron normalmente leves.

1. INTRODUCTION

Poultry farming has experienced a strong development since the second half of the twentieth century. This development led to the intensification of animal production systems, maximizing the productivity of farms and reducing production costs. Consequently, market prices for poultry products decreased, resulting in an economic benefit for consumers.

A good example is the case of Spain: In 1961, intensive rearing poultry facilities in this country accounted for 34 million of places, 79.8 millions of animals slaughtered and a production of 79,100 tons of poultry meat. In 2009, these figures were considerably higher: 138 million places, 658 millions of animals slaughtered and 1,179,470 tons of meat production (FAOSTAT, 2011). The economic benefit for the consumer can be demonstrated with the percentage of an average Spanish salary spent on food: in 1958, it was 55.3%, while in 2002 it had decreased to 17.8% (INE, 2004).

This intensification was based on genetic selection, improvement of feed strategies and modernization of poultry houses which incorporated new automatized systems for lighting, feeding, environmental control and management (Havenstein *et al.,* 2003a,b). Broilers chickens have been submitted to an intensive genetic selection that increased their growing rate and made them the fastest growing farmed species (Meluzzi and Sirri, 2008). For example, slaughter age in broilers has been reduced by about 1 day per year in the latest 30 years of the 20th century (Schultz and Jensen, 2001) and compared to its wild ancestor, the red junglefowl (*Gallus gallus*), adult broilers grow to more than four times the body mass of their wild predecessor (Jackson and Diamond 1996).

However, during this intensification of production systems, little attention was paid to the impact on health and welfare of the animals, leading to the increase of several metabolic, locomotive or behavioural disorders (Bessei, 2006) and, therefore, to a worsening of the welfare status of the birds (SCAHAW, 2000). Nowadays, society places new demands on farmers, asking to improve food safety and quality of final livestock products, ensuring animal welfare and low environmental impact (Meluzzi and Sirri, 2008).

There is not a universal definition for animal welfare because it is difficult to set this concept and many people have their own opinion. The following are some of the definitions more generally accepted by the international scientific community:

- The Farm Animal Welfare Council (2009) defines animal welfare as five freedoms: *1. Freedom from thirst, hunger and malnutrition; 2. Appropriate comfort and shelter; 3. Prevention or rapid diagnosis and treatment of injury and disease; 4. Freedom to display most normal patterns of behaviour; and 5. Freedom from fear.*
- In accordance with this, the scientific community seems to agree that animals should be housed in comfortable places, with access to good feeding, being able to express an appropriate behaviour and enjoy good health (Welfare Quality®, 2009).
- Broom (1986) and Manteca (2001) approached the issue from the point of view that welfare of an individual is its state as regards its attempts to cope with its environment.

As there can be found different definitions for animal welfare, there are different indicators which can be used to assess it, such as health, mortality or productivity measures (Broom, 1991; Torres, 2001). Although there is a direct relationship between productivity and animal welfare, it is well known that animals are also able to have high productions under severely restricted welfare conditions. Therefore, despite a decrease in productivity may be indicative of poor welfare, maximum productivity is not indicative of maximum welfare (García-Belenguer, 2001).

One of the main contributors to welfare of broiler chickens, in commercial farm conditions, is the quality and status of litter because they usually spend their entire life on it. Besides, litter affects the environmental conditions of the building by influencing dust and ammonia levels in the air which, in turn, may affect health of the birds leading mainly to respiratory problems. Furthermore, litter also has a direct influence on the skin condition of the birds, being wet litter a major risk factor for contact dermatitis (SCAHAW, 2000).

Litter consists, essentially, of the material used for bedding mixed with excrements, feathers, remnants of feed, skin and moisture (Ritz, 2004).

The main component is the bedding material. According to the Cobb management guide (Cobb Vantress Inc., 2008), it should be absorbent, non-dusty, lightweight, inexpensive, non-toxic and useful as fertilizer. Bedding material has several important functions as absorbing moisture, absorbing and diluting faecal material and isolating birds from the cooling effect of soil. Typical Spanish intensive broiler farms use different bedding materials according to the availability and market prices. In Spain, litter is generally removed at the end of each cycle before cleaning and disinfecting the building; On the contrary, in some other countries as the United States, litter is generally reused in consecutive flocks (Calvet, *et al*., 2011).

Excrements of birds are other important component of the litter. The accumulation of excrements leads to an increase of pH, moisture and nitrogen content of the litter (Ritz, 2004).

Litter moisture content should not exceed 35% (Cobb Vantress Inc., 2008); however, around the drinkers it is common to find humidity levels up to 70% which may produce wet crusty litter or caked litter (Meluzzi and Sirri, 2008). High litter moisture has an undesirable effect on health and welfare of the chickens since positive correlations have been reported between litter moisture and foot pad dermatitis (FPD), hock burns (HB) or breast blisters (BB) (Harms *et al*., 1977; Algers and Svedberg, 1989; Ekstrand *et al*., 1997). FPD, HB and BB may be summarized under the syndrome “contact dermatitis”, characterized by pain, inflammation, hyperkeratosis and necrosis of the affected tissues. Additionally, growth rates may be also affected due to pain-induced inappetance (Martland, 1984, 1985; Ekstrand *et al.,* 1997.) and secondary infections may further worsen the condition of the birds (Meluzzi, 2008).

Litter characteristics are also related to the air quality within the farm due to the production and volatilization of ammonia as well as the emission of particulate matter to the environment.

Environmental ammonia inside broiler houses arises from the microbial breakdown of uric acid of the excrements (Carlile, 1984). The efficiency of this conversion is affected by different factors as temperature, PH and moisture of the litter, properties of bedding material or ventilation flow and management techniques (Elliott and Collins, 1982; Patterson and Adrizal, 2005). Increased moisture levels promote proliferation of microorganisms in the litter, increasing the production and volatilization of ammonia (Groot Koerkamp *et al.,* 1999; Al Homidan *et al.,* 2003; Oviedo, 2005).

Due to the volatile and water-soluble nature of ammonia, it can be dissolved into the mucous membranes of the respiratory epithelium and eyes of animals, being responsible for the onset of sneezing, dyspnoea, inflammation of the air sacs, respiratory diseases and keratoconjunctivitis (Carlile, 1984). Further investigations suggested that lung diseases, as well as inhalation of airborne irritants such as ammonia, result in reduced pulmonary gas exchange causing also an exacerbation of ascites (Charles and Payne, 1966). Indeed, Scheele *et al.,* (1991) reported that broilers with respiratory infections are more susceptible to ascites and have decreased capacities for O_2 consumption when compared with their disease-free counterparts. Some studies even reported higher mortality and lower feed consumption (Carlile, 1984; Miles, 2004), lower vaccine response (Caveny, 1981) or increased disease susceptibility (Beker *et al,* 2004). Therefore, high levels of ammonia in farm inner environment may have a negative effect on animal health, reducing also, the performance of broilers (Kristensen and Wathes, 2000; Miles *et al.,* 2002, 2004; Beker *et al*, 2004).

Because of the negative effects of ammonia, the Animal Welfare Commission of the European Union settled, on the EU Directive 2007/43 EC a maximum ammonia concentration of 20 ppm at the head of the chickens. However, Carlile (1984) showed that in conventional farms, commonly, chickens are housed in facilities with 50 ppm of ammonia and are challenged occasionally by peaks of up to 200 ppm under conditions of poor ventilation. In 1998, Groot Koerkamp detected several poultry houses with average ammonia concentrations between 20 and 30 ppm with instant variations levels far above of the 20 ppm threshold.

Another known air pollutant from the litter is the particulate matter (PM). It is defined as a complex mixture of suspended particles with different physical, chemical, and biological characteristics, which determine both its behaviour, as well as its environmental and health effects (EPA, 2004). PM from poultry houses largely comes from the litter (Aarnink *et al*., 1999; Cambra López *et al.,* 2010). Some bedding materials or the status of the litter, among other factors, may increase the PM levels on the environment (Shanawany, 1992; Kaliste *et al*., 2004).

Different conventions are used to classify PM. Occupational health sizes are defined by the International Standards Organization, in ISO 7708 (ISO, 1995), and the European Standardization Committee, in EN 481 (EN, 1993). Occupational health sizes are based on the behaviour of particles in the human respiratory tract, and are derived from the depth of entrance into it. Human health-related sizes according to these conventions are:

1. Inhalable: particles which can be inhaled through the nose and mouth; *2. Thoracic:* particles inhaled which can penetrate into the larynx; and *3. Respirable*: particles which can go beyond the larynx and penetrate into the unciliated respiratory system (EN, 1993). On a similar way, the US EPA Code of Federal Regulations (US EPA, 2001) and the Council Directive 1999/30/EC defined PM as two different fractions: *PM10* and *PM2.5*, according their pass through a size-selective inlet with 50 % efficiency. Based on these classifications, occupational health size fractions can be compared with US EPA fractions: PM10 is comparable to the thoracic fraction, although with differences in the range of particle. The PM2.5 fraction can be considered equivalent to the high risk respirable defined by the ISO 7708 (ISO, 1995). The respirable fraction is comparable to PM4.

PM traditionally has been regarded as a pollutant causing detrimental effects on animal performance and efficiency (Donham and Leininger, 1984; Al Homidan and Robertson, 2003). It can cause respiratory problems in humans and animals (Zuskin *et al.*, 1995; Donham, 2000; Radon *et al.*, 2001). PM can adsorb gases and odorous compounds or transport air-borne potential pathogens, enhancing its biological effect (Cambra-López *et al.*, 2009). Likewise, emission outdoors of PM can promote the spread of the pathogen attached to particles and, consequently, the transmission of diseases among farms. Furthermore, PM has an increased importance in broiler production since chickens have not diaphragm and consequently are not able to expulse any inhaled particle by coughing.

Therefore, is no difficult to notice that composition and status of litter is a factor with direct influence on air quality within farms and on air pollution, mainly caused by emissions of harmful gases and PM (Weaver and Meijerhof, 1991; Patterson and Adrizal, 2005; Bessei, 2006).

Consequently, it can be said that litter has a direct effect on animal welfare and health (Al Homidan *et al.*, 2003), performance of broilers and carcass quality (Martland, 1985). Thus, the implementation of new management technics, aimed to maintain the optimal conditions in bedding material as long as possible during the broilers rearing cycle, is needed. These techniques should help to prevent the onset of other health and welfare problems

Litter aeration (LA) is a manure management method aimed to reduce litter moisture content and anaerobic decomposition (ASABE, 2007). Therefore, it has also been studied for its capacity to reduce the concentration of ammonia in poultry facilities and to increase productivity, improving litter characteristics (Van Middelkoop, 1994, Allen *et al.,* 1998). During the production cycle of broilers, LA procedures can be used as an alternative to prevent cake formation and, consequently, mitigate contact dermatitis on pads and hocks. These potential benefits must be confirmed, as well as the impact that this practice can have on the concentration of airborne harmful compounds and emissions (gases, PM and microorganisms).

The main aim of this study was to evaluate the effect of LA in the course of a broiler production cycle on:

- Litter physic-chemical characteristics, considering moisture, ash content, nitrogen content and pH.
- Environmental conditions affecting animal welfare such as ammonia and particulate matter concentrations.
- Animal welfare, considering different approaches: productive parameters, mortality and lesions prevalence and severity.

2. MATERIALS AND METHODS

2.1. Animals and housing

This experiment was carried out from October, 28th to December, 9th 2010 in the poultry meat facilities of the Animal Technology Centre (CITA-IVIA) located in Segorbe (Castellón, Spain). Three identical experimental rooms (Room 1, 2 and 3; 13.22 x 5.9 m.) were used for this purpose (Figure 1).

The concrete floor of the rooms was covered with a 10 cm depth wood shavings litter. Two of the rooms were subjected to LA (1 and 3) and the other one (2) was used as control (C) room. The first day of the experiment, the three rooms were filled with 800 one-day-old Cobb 500^{TM} male chicks. The animals were reared during a 42-day cycle.

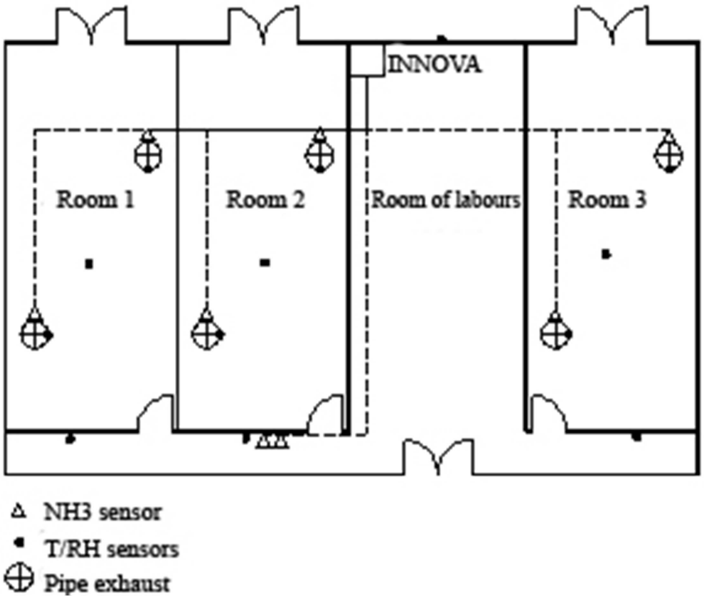

Figure 1: Premises and equipment used during the experimental period

Housing conditions simulated those found in most commercial farms. Each room was equipped with 22 feeders and 111 drinkers (distributed in 2 and 3 lines, respectively).

An automatic environmental control system (COPILOT, France) was used for the environmental control. Temperature and relative humidity (RH) were maintained according to breeder's recommendations (Cobb Vantress Inc., 2008) and lighting regime varied gradually from a 23:1 scheme (23 hours of light and 1 hour of darkness) during the first three days to a 16:8 scheme.

Feed and water were provided *ad libitum* throughout the experiment. Two different types of feed were used: starter feed, used from day 0 to day 21 and grower feed, from day 21 to day 42.

LA was carried out weekly from the third week of the rearing cycle by using a machine designed for this purpose (Benza, ER73AV, Spain) shown in figure 2.

Figure 2: Machine used for litter aeration and detail of the rotative parts

2.2. Litter characteristics

Samples of the litter were taken weekly from each room, according to the protocol proposed by Tasistro *et al.*, (2004) and were analysed for total Kjeldahl nitrogen (TKN), pH, dry matter (DM) and ash content.

On the other hand, in order to assess the stratification of moisture content and depth of the caked litter, compound samples were taken weekly from each room. These samples were collected from 18 points around the feeders and drinkers and from the central corridors between feeding and drinking lines. Sampling was separately performed for the highest and lowest centimetres of litter.

2.3. Environmental conditions

The management of ventilation and heating was similar to that used on conventional farms. Ventilation rate was monitored continuously in the three rooms by installing fan wheel anemometers (EXACTFAN 56, Exafan, Spain) in one of the exhaust pipes of each room. Temperature and RH were also recorded by installing three sensors (Onset

HOBO U12, Pocasset, USA) inside of each room and installing other sensors outside, both at the entrance of air into the rooms and out of the facilities (Figure 1).

Throughout the experiment, ammonia concentrations were measured every two hours in eight different points: two in the air exhausts exit of each room, and two on the outside using a photoacoustic measurer (INNOVA-1412, Lumasense, Denmark). The gas sample was transported from into the room to the measurer using polytetrafluoroethylene (PTFE) conductions.

Concentrations of PM in each room were recorded with two systems: a continuous, Tapered Element Oscillating Microbalance (TEOM® 1405-D model, Thermo Fisher Scientific, USA) that simultaneously measured two fractions of particles (PM10 and PM2.5) and a gravimetric point registration system, using a cascade impactor (RespiCon®, HundWetzlar, Germany) which separated the total particles (TSP) and the earlier fractions (PM10 and PM2.5) in filters that were weighed before and after sampling with a resolution of 10 mg, under conditions of controlled temperature and humidity.

2.4. Animal welfare and lesions assessment

Feed and water consumption were measured weekly recording feed supplied and the remaining feed in the hopper and feeders. Water consumption was monitored by water flow counters fitted on the water supply of each room. In addition, the same day when the feed was weighed, 50 animals randomly selected were taken from each room in order to be weighed to monitor their growth. Food conversion rate (FCR) was calculated by dividing the average accumulated feed consumption at day 42 by the average body weight of the birds at that same age. Similarly, the water:food rate, resulted of dividing the accumulated water consumption by the accumulated feed consumption, both values at the 42nd day of life of the birds. The mortality rate of the animals was recorded daily.

FPD, HB, BB and conjunctivitis (CJ) were also evaluated in those animals. FPD, HB and BB were assessed using the score proposed by Welfare Quality (2009 CJ was evaluated in live animals according to the protocol proposed by Beker *et al*., (2004). However, since the majority of the affected animals were scored as 1, the data were summarized as presence/absence of the lesion.

Tonic immobility (TI) is defined as an unlearned response characterized by a catatonic-like state of reduced responsiveness to external stimuli induced by a brief period of physical restraint (Jones, 1990). It has been induced in a wide variety of species, including fishes, reptiles, amphibians, birds and mammals. TI is a reliable measure of fearfulness in chickens, extensively used in that sense (Jones, 1986). In this experiment, TI was assessed on day 41 in forty animals from each room. As soon as the broiler was caught, TI was induced in a nearby room by inverting the bird on its back with its head hanging over the edge of a U-shaped wooden cradle (Figure 3).

Figure 3: U-shaped wooden cradle and details of the tonic immobility induction procedure

The bird was restrained for 15 seconds by placing one hand on the sternum while covering the head with the other hand, according to the procedure described by Jones & Faure (1981) with the observer sat in full view of the chicken and at a distance of about 2 meters from the bird. If the chicken remained immobile for a period of 10 seconds after the experimenter removed his/her hands, the time until the bird showed a righting response was recorded. If the bird showed no righting response over a 15 minutes period, the session was ended and a maximum score of 15 minutes (900 seconds) was assigned (Stub & Vestergaard, 2001). On the contrary, if the bird righted itself in fewer than 10 seconds, then it was considered that TI has been not induced and the restraint procedure was repeated. The number of attempts needed to induce TI for at least 10 seconds was recorded and if TI was not induced after five attempts, the bird was deemed not to be susceptible and its TI score was 0 seconds (Bizeray *et al.*, 2002).

The following day (day 42), the same animals were collected again, humanely culled, weighed and examined in search of respiratory tract and thoracic air sacs lesions, heart abnormalities, hidropericardium and ascites according to the protocol proposed by Terzich *et al.* (1998). The heart of culled birds was weighted in order to detect hypertrophic changes and other alterations. Likewise, the birds were inspected to assess corneal ulcer and tibial dyschondroplasia (TD).

Corneal ulcer is a lesion usually caused by an infection or by a wound; it has been used as welfare indicator (Olanrewaju *et al.,* 2007). In this investigation, it was assessed staining the corneal epithelium with fluorescein.

TD is another health parameter that is commonly included in investigations related to the welfare of broilers as it can lead to impaired movement compromising reaching feed and water (SCAHAW, 2000). The chickens were checked for TD when slaughtered at 42 days of age in accordance with the procedure proposed by Stub & Vestergaard, (2001). However, as in the case of BB, HB, FPD and CJ; the results were summarized as presence/absence due to the low incidence of lesions scored higher than 1.

2.5. Statistical analysis

Since no significant differences were found between rooms of the same treatment for any factor, therefore the factor room was not included in any model. All these analysis, were performed per weeks, in order to detect more accurately the differences between treatments, minimizing the effect of the age of the animals.

Data of litter characteristics (DM content, ash, TKN or pH) was subjected to analysis of variance using the GLM procedure of Statgraphics© Centurion XV software (Statpoint, 2006). Treatment was the only factor included in the statistical model which is the same for all the different dependent variables, as follows:

$$\boldsymbol{X_i = \mu + T_i + err}$$

Where: X_i*: dependent variable (DM content, ash; TKN or pH);* μ*: mean of the studied variable;* T_i*: treatment i (LA or C); err: random error.*

Stratification of moisture in the litter was analysed using the GLM procedure of the same statistical package. The model used in this case included the factor “type of sample”, as follows:

$$\boldsymbol{DM_{ij} = \mu + T_i + Tp_j + T_i * Tp_j + err}$$

Where: DM_{ij}*: dependent variable (DM content);* μ*: mean of the studied variable;* T_i*: treatment i (LA or C);* Tp_j*: type of sample j (deep or surface); err: random error.*

Body weight, feed consumption, TI, difference of temperature after TI induction and heart weight were analysed using the GLM procedure of the previously cited software. Additionally, in order to be allowed to use the GLM procedure, data TI duration had to be converted into a normal distribution using a natural log transformation. The following model was applied for all those variables:

$$\boldsymbol{Y_{ij} = \mu + T_i + err}$$

Where: Y_{ij}*: dependent variable (Body weight, feed consumption, TI, difference of temperature after TI induction and heart weight);* T_i*: treatment i (LA or C);* R_j*: room j (1, 2 or 3); err: random error.*

Finally, categorical data as the presence/absence of lesions, number of attempts for inducing TI and mortality were studied using a Chi square test, again with Statgraphics© Centurion XV.

Mean values were reported as mean ± standard error (s.e.) and they were separated using Fisher's Least Significant Difference (LSD) comparisons. Differences were considered significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Litter Characteristics

The accumulation of excrements increased pH levels, ash, moisture and nitrogen content of the litter, as stated by Ritz (2004).

DM content did not present statistical differences between treatments at any week. However, observed values were constantly higher for the C group, contradicting the expected hypothesis that LA would decrease litter moisture content in the litter (Fig. 4). At the end of the cycle, DM values were superior to those recorded by Meluzzi *et al.* (2008) and Martland (1985), although the latter example was an experiment carried out with broilers reared during 9 weeks to a comparable final body weight.

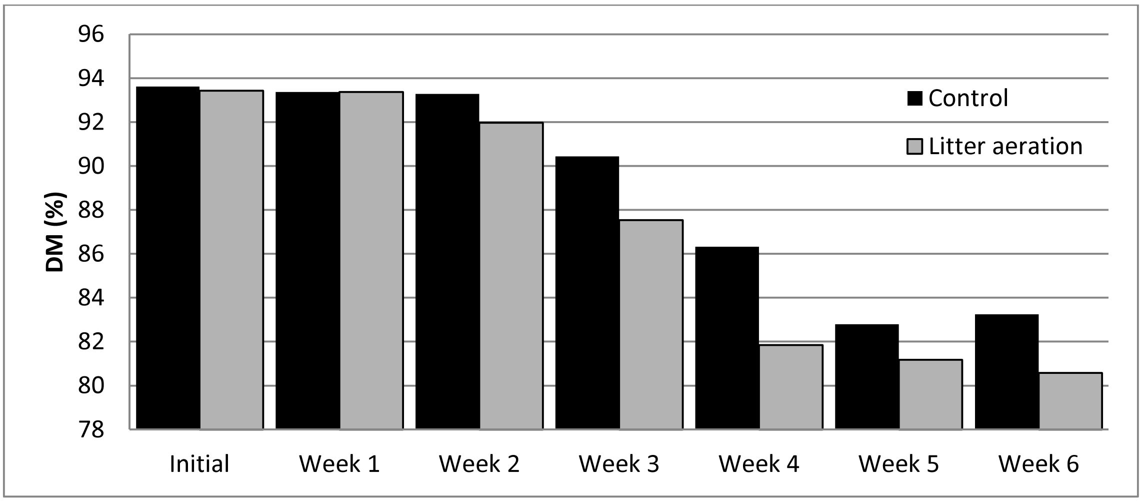

Figure 4: Evolution of dry matter content in the litter during the rearing cycle, in both treatments

Regarding the stratification of litter moisture, at the beginning of the experiment, no significant variation was observed between treatments for DM content neither in surface samples (92.3% and 92.1% for C and LA groups, respectively) nor in deep samples (93.4% for C group and 92.7% for LA group). Likewise, there were no differences in the last week of the cycle neither in surface samples with 73.9% for both treatments, nor deep samples (87.3% and 81% for C and LA groups, respectively). On the other hand, when considering differences between types of samples (surface or deep), similar values were measured for both types in the first week of the experiment but statistically significant differences arisen at weeks 3 (p= 0.0127), 5 (p= 0.0493) and 6 (p= 0.0325), as reflects the figure 5.

These results indicate that moisture tends to be distributed into the superficial parts of the litter, but no effect of the treatment is observed.

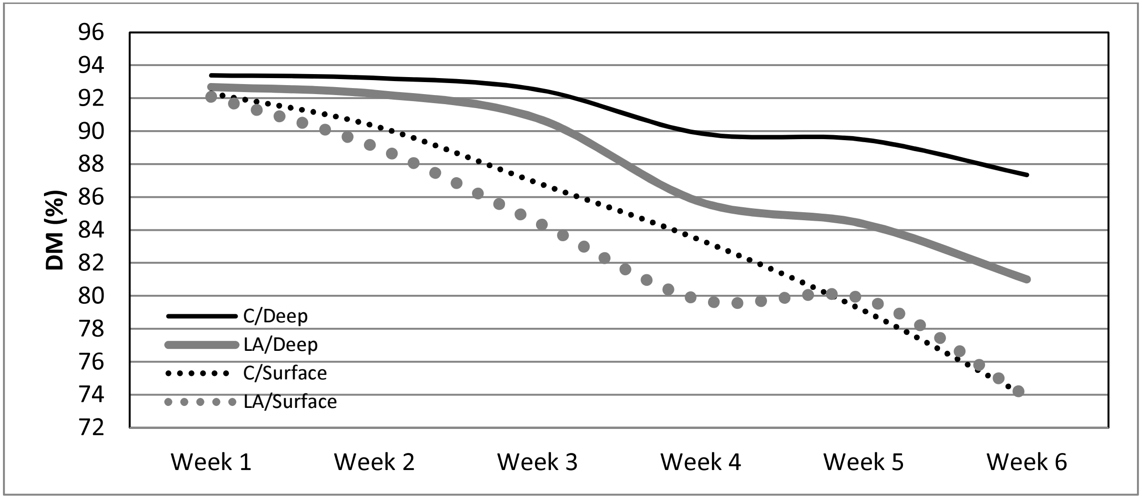

Figure 5: Dry matter content (%) in surface and deep samples in both treatments

The average total Kjeldahl nitrogen (TKN) content was slightly higher in rooms where the LA was performed, which could lead to a higher ammonia formation, as dicussed later. However, this parameter showed no clear trend and no statistical significance was found except in the fourth week (p=0.0157), as seen in figure 6.

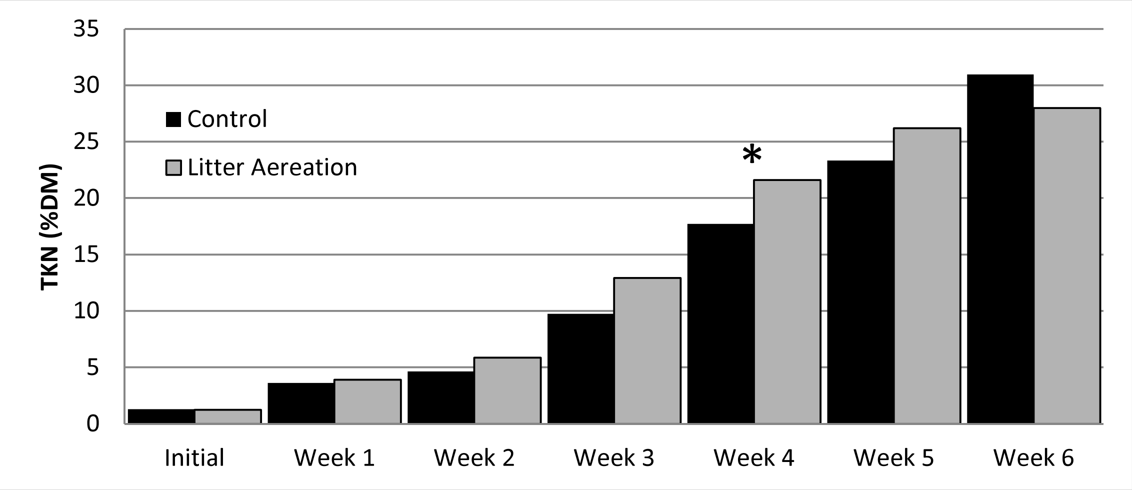

Figure 6: Evolution of TKN content (as % of DM) in the litter during the cycle for both treatments. (Asterisks indicate that the referred pair of means is statistically significant at the 95.0% confidence)

The PH level increased throughout the rearing cycle in both treatments, probably due to the accumulation of poultry excrements, which pH is typically between 7.5 and 8.5 (Ritz, 2004). At the beginning of the experiment there appeared significant differences between groups (p= 0,0269) but from there on, statistical significance is presented no more (figure 8). Despite this fact, since week 4, differences are bigger than 0.5 points; this could have implications in the process of ammonia formation, which is negligible when litter pH is lower than 7 and high when a pH of 8 is reached (Reece *et al.*, 1980; Elliot and Collins, 1982; Carr *et al.*, 1990).

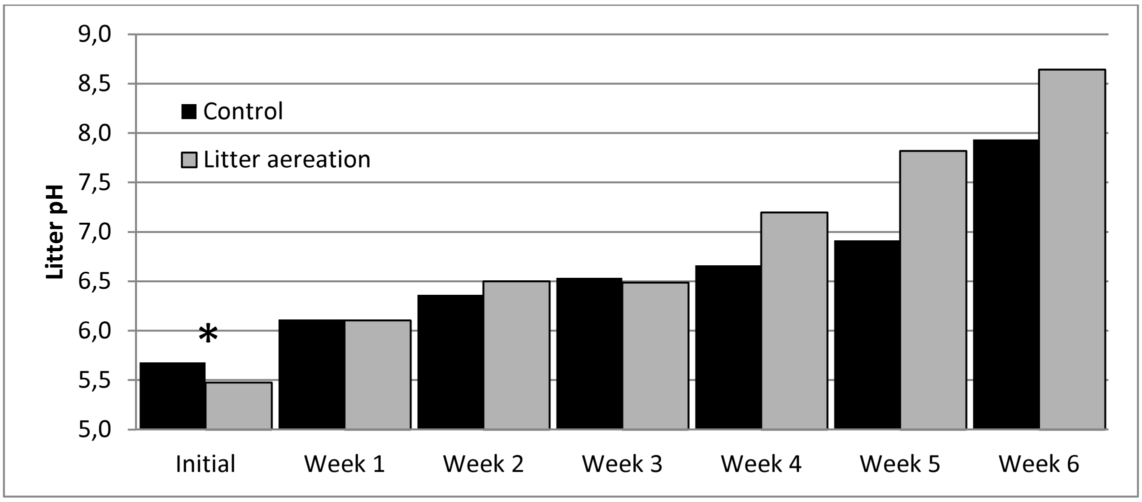

Figure 7: Evolution of litter pH during the rearing cycle for both treatments. (Asterisks indicate that the difference between the referred pair of means is statistically significant at the 95.0% confidence)

With regard to ash content (figure 6), was also slightly higher in C room although no statistical differences were found except for week 5 (p=0.0302).

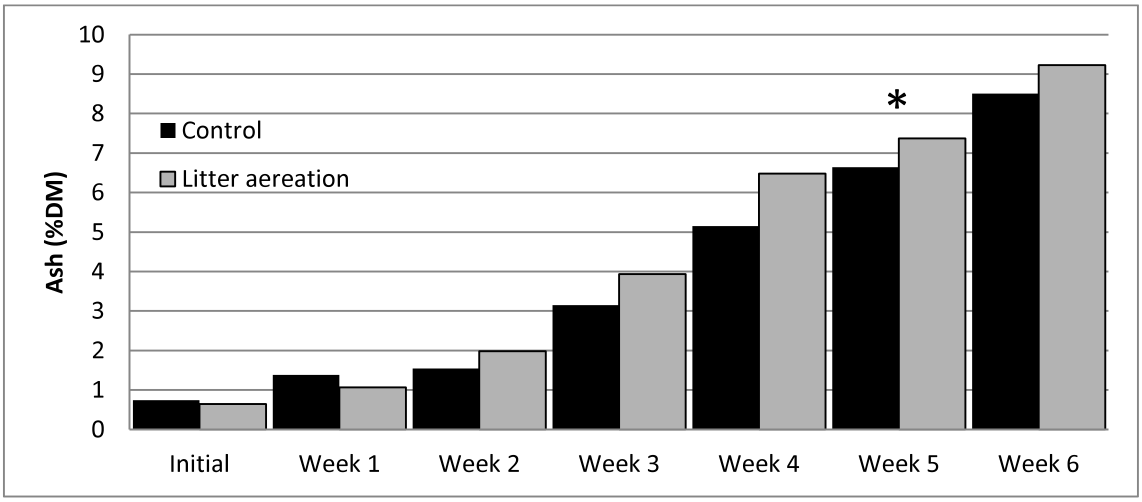

Figure 8: Evolution of ash content of the litter (as % of DM) during the rearing cycle for both treatments. (Asterisks indicate that the pair of means is statistically significant at 95.0% confidence)

3.2. Environmental conditions

The recorded values were very similar for both treatments and laid within the range recommended by the breeding company (Cobb Vantress Inc., 2008).

The average daily temperatures (mean ± s.e.) registered inside C and LA group were 26.5±0.5 ºC and 26.5±0.3 ºC, respectively. The average daily HR (mean ± s.e.) registered for C and LA groups respectively, were 37.2±0.3 % and 39.3±0.2 %. The following figure shows the average daily values of temperature and relative humidity (RH) for each group registered during the experimental period.

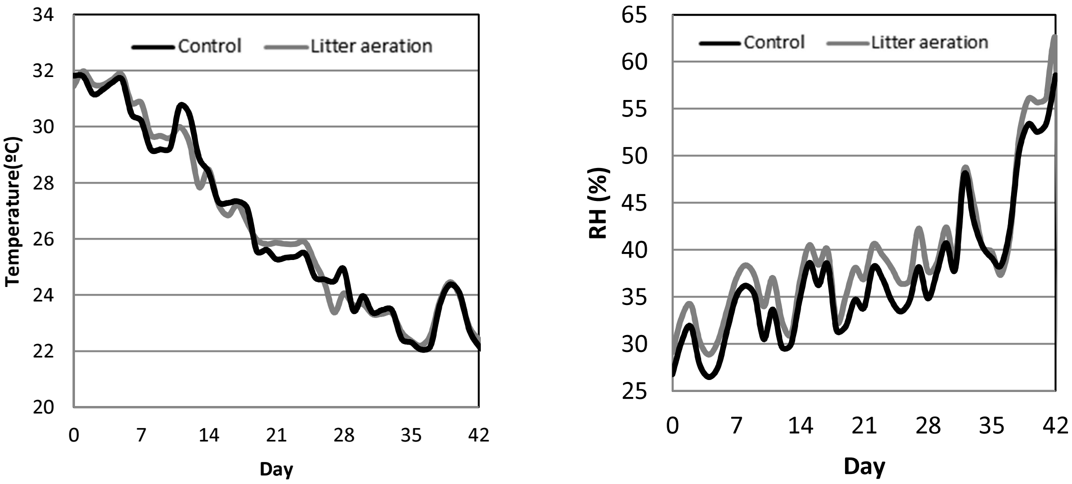

Figure 9: Daily average temperature and RH for each treatment during the rearing cycle.

In comparison with the data obtained by Calvet *et al.* (2011) in winter conditions on a commercial farm in the southeast of Spain, indoor average (mean ± s.e.) temperature and RH values were lower (temperature: 26.9±0.1 ºC; RH: 57.1±0.4 %). Indoor temperature average values did not differ considerably from those reported by Seedorf *et al.* (1998), who found an average temperature of 25.3 °C in summer and 24.5 °C in winter on several farms in Europe. This uniformity is probably due to the standard conditions in which these animals are generally reared.

The average daily concentration of ammonia (mean ± s.e.) in the C room (1.15±0.05 mg/m^3) was lower than in the rooms where LA was performed (2.20±0.10 mg/m^3). Taking into account that ventilation rates were similar for both groups, this might be explained by an increased ammonia emission, higher in LA group throughout the cycle perhaps due to a higher pH and TKN content in the litter, plus the oxygenation of the litter induced by LA procedures. Figure 10 shows the ammonia concentrations registered during the experimental period in the C and LA groups.

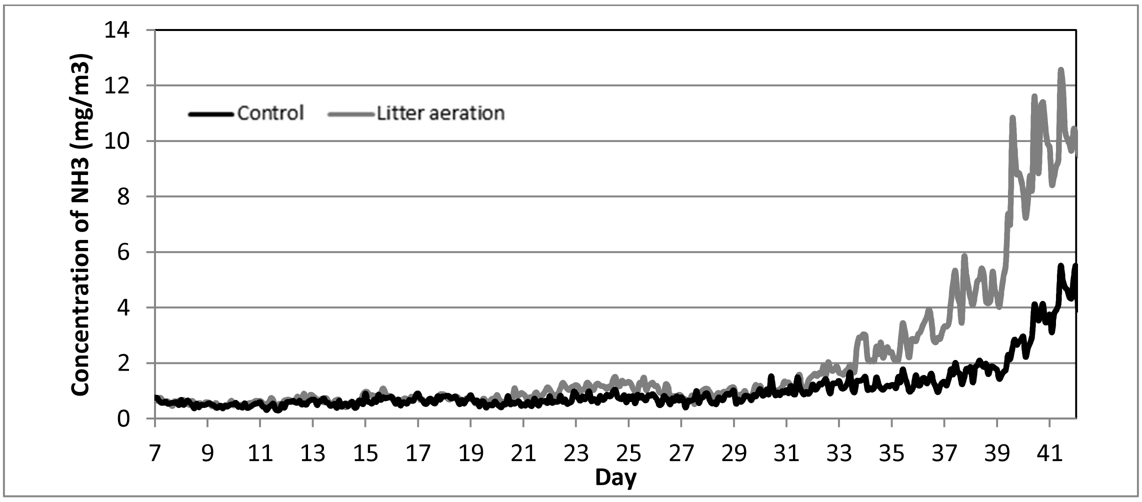

Figure 10: Average daily ammonia concentrations for each treatment during the rearing cycle

Ammonia levels were lower than those found by Calvet *et al*. (2011) in a commercial farm in winter conditions. These might be due to some differences found between the experiments, as a lower stocking density, a higher DM content of the litter or a higher ventilation rate in the present investigation.

These concentrations were below the threshold which may affect human wellbeing and welfare and productive parameters in broilers, settled at 17 mg/m^3 (25 ppm) of ammonia by Al-Homidan *et al.* (2003), Carlile (1984) and the CIGR (1992). However, ammonia levels exceeded 10 ppm (6.95 mg/m3), the upper limit recommended by the Cobb management guide (Cobb-Vantress Inc., 2008). Additionally, at the end of the cycle, ammonia concentrations in LA group occasionally exceeded 14 mg/m^3 (20 ppm) which is the maximum value settled by the Spanish regulation RD 692/2010 of June, 3rd 2010 (at peaks times of days 39 and 42, reached 15.49 and 16.12 mg/m^3, respectively).

A distinctive feature of ammonia concentrations pattern was that it started to increase sharply at the end of the cycle. This could be because of an increment in ammonia production as litter pH approaches 7.0 (Reece *et al.*, 1980; Elliot and Collins, 1982; Carr *et al.*, 1990). Besides, as environmental RH rises, ammonia levels may also increase (Weaver and Meijerhof, 1999). In accordance with these facts, during this experiment, TKN content, moisture and pH in the litter and environmental RH continuously augmented, reaching optimal values for microbial ammonia production at the end of the cycle and taking place, consequently, the peak of environmental ammonia.

On the other hand, this harsh increase of environmental ammonia did not take place at the very same moment in both groups: it was around the 5th and 6th week in LA and C groups, respectively. The earlier onset of this peak of ammonia in the LA group could be explained by a constant higher litter pH (which exceeded 7.0 at 5th and 6th week in LA and C groups, respectively), a higher TKN content, plus the weekly oxygenation induced by the LA procedures performed in this group.

In relation to the particulate matter (PM), average concentrations of PM throughout the cycle were higher in LA group for all different fractions: 4.36 mg/m^3 (LA group) and 3.79 mg/m^3 (C group) for TSP; 2.05 mg/m^3 (LA group) and 1.53 mg/m^3 (C group) for PM10; and 0.28 mg/m^3 (LA group) and 0.17 mg/m^3 (C group) for PM2.5. Additionally, the most characteristic features of PM concentrations pattern were the peaks occurred in LA group after LA procedures, as shown in figure 11.

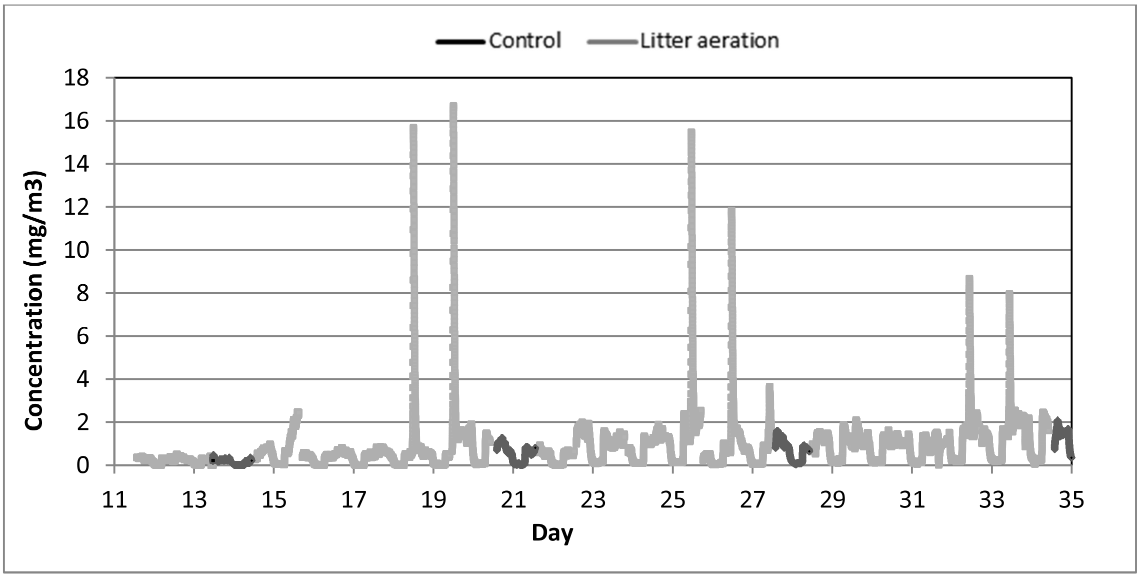

Figure 11: Evolution of the hourly concentration of particulate matter (PM10) from day 11 to 35

The average concentrations of PM obtained in this study were in accordance with most of the results recorded by other authors in similar poultry rearing conditions (Table 1). However, as can be seen in figure 11, most them were widely exceeded at peak times, especially during the LA procedures, when PM10 concentrations were occasionally higher than 16 mg/m^3.

	Concentration (mg/m^3)		Country	Source
	Mean	**Range**		
Inhalable PM	10.1	-	England	Wathes *et al*. (1997)
	-	9.2-11.1	Scotland	Al Homidan *et al.* (1998)
	-	1-14	Germany	Hinz and Linke (1998)
	7.15	3.83-10.36	England, The Netherlands, Denmark and Germany	Takai *et al*. (1998)
	3.21	-	The Netherlands	Aarnik *et al.* (1999)
	-	8.2-9.0	The Netherlands	Ellen *et al.* (1999)
	-	.73-11.39	U.S.	Redwine *et al.* (1987)
	-	1.77-4.41	Scotland	Al Homidan (2004)
	4.32	2.27-8.58	Australia	Benhazi *et al.* (2008)
	-	2.0-4.9	Croatia	Vucemilo *et al.* (2008)
Respirable PM	5.43 (28th day)	-	U.S.	Willis *et al.* (1987)
	9.71 (49th day)			
	0.10	-	England	Wathes *et al.* (1997)
	0.81	0.42-1.14	England, The Netherlands, Denmark and Germany	Takai *et al.* (1998)
	-	1.4-1.9	The Netherlands	Ellen *et al.* (1999)
	0.84	0.30-1.80	Australia	Benhazi *et al.* (2008)

Table 1: Review of measured inhalable and respirable PM in broiler houses with litter in chronological order of publication (Cambra López *et al.*, 2009).
[Note: inhalable PM is considered equivalent to TSP, and respirable PM in these cases is used equivalent to TSP, and respirable PM in these cases is used equivalent to PM4 (EN 481:1993; ISO 7708:1995)]

The concentrations of PM also overpassed many other recommendations as:

- The Cobb management guide (Cobb Vantress Inc., 2008) recommends do not exceed 3.4 mg/m^3 of respirable PM.
- Legally binding workplace exposure limits in the United Kingdom are: 10 mg/m^3 for inhalable PM and 4 mg/m^3 for respirable PM, for an 8-h average. For short term exposure (15 min), exposure limit is 20 mg/m^3 for inhalable PM (HSE, 2007).
- German Ordinance on Hazardous Substances (GefStoffV) established the short term (15 min) workplace exposure limits in 10 mg/m^3 for inhalable PM, and 3 mg/m^3 for respirable PM (BGIA, 2009).
- The CIGR, (1992), established recommended limits for animals in 3.4 mg/m^3 for inhalable PM, and 1.7 mg/m^3 for respirable PM.

- European Directives Council Directive 1999/30/EC and 1996/62/EC, settled the daily limit value for PM10 in 50 mg/m^3, not to be exceeded more than 35 days per year, and the annual average limit in 40 mg/m^3.
- The Air Quality Directive (2008/50/EC), in order to protect human health and the environment, has set an annual average limit for PM2.5 of 25 mg/m^3. It has also settled an annual exposure concentration obligation of 20 mg/m^3, based on an average exposure indicator measured on three consecutive years.

Moreover, it has been described a positive relationship between the concentration of this pollutant and mortality in chickens (Guarino *et al.*, 1999). Workers of poultry farms exposed to average concentrations of PM above the limits of 2.4 mg/m^3 of TSP and 0.16 mg/m^3 of respirable PM (PM4, particulate matter with 4 microns in diameter or less) have been associated with lung problems (Donham *et al.*, 2000). Just *et al.* (2009), also identified a higher prevalence of respiratory problems in workers of poultry farms in comparison with other production systems due to higher concentrations of PM.

3.3. Animal welfare: production parameters, mortality and health indicators

The animals started the experiment with a similar average body weight (mean ± s.e.): 84.49±0.35 g. for the C group and 84.18±0.25 g. (p=0.49). for the LA group; However, they finished the cycle with 3,192.8±25.9 g. and 3,110.6±18.1 g. for the C group and the LA group, respectively, existing statistical significance between treatments (p=0.0107).

Final body weight was higher than the results observed by Meluzzi *et al.* (2008) in 43-days-old male broilers; Calvet *et al.* (2009) in 49-days-old, both male and female broilers; and Sirri *et al.* (2010) with male broilers of the same age. However, final body weight was lower than the results published by Meluzzi *et al.* (2008) in 49-days-old male broilers.

According to the proposed model, statistical significance arises from the third week for the factor “Treatment” as seen in table 2. From there on, poultry chickens of C group appear to be heavier than chickens from LA group. According to these results, LA seems to have an unfavourable effect on the body weight of the broilers.

Week	Treatment	
	C	LA
0	84.5±0.3	84.2±0.2
	0.4893	
1	141.5±1.7	140.0±1.2
	0.485	
2	387.2±4.1	378.6±2.9
	0.094	
3	805.3±9.3	774.1±6.5
	0.007*	
4	1459.2±15.1	1418.2±11.1
	0.0308*	
5	2255.6±21.9	2190±15.7
	0.0165*	
6	3192.8±25.9	3110.6±18.1
	0.0107*	

Table 2: Evolution of weekly average weight: mean ±s.e. (in grams) and p-values for the different factors of the proposed model. (Asterisks indicate statistical significance at the 95.0% confidence)

With regard to average daily feed intake, it was higher in LA group since week 3, although no statistical significance was found. It was 84.9±24.86 g. and 97.57±20.55 g/bird for C and LA groups, respectively (mean ± s.e.). Figure 13 shows the evolution of daily feed intake depending on the treatment during the experiment.

Consequently, accumulated feed consumption at the end of the cycle was higher for LA group: 3,568 g/bird and 4,098 g/bird for C and LA groups, respectively. These values were lower for the C group and higher for the LA group than those reported by Sirri *et al.* in 2010; they were lower than the total feed intake observed by Meluzzi *et al.* (2008). Additionally, these values were lower than the expected values of accumulated feed consumption recommended by the breeder company for 42-days-old male broilers.

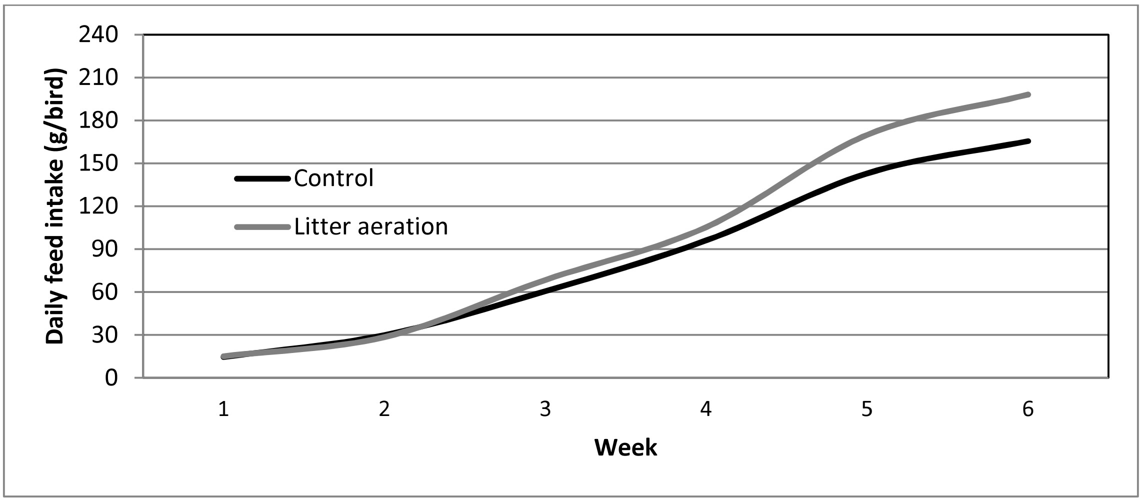

Figure 12: Evolution of average daily feed intake per bird, during the cycle for both treatments

At the end of the experiment, as consequence of a higher feed consumption and lower growth, the food conversion rate (FCR) was higher in the animals from LA group (1.15 and 1.35 for C and LA groups, respectively). These values were lower than those recorded for male broilers of similar genetics (Havenstein *et al.,* 2003). These data is also lower than the FCR observed by Calvet *et al.* (2004), Meluzzi *et al.* (2008) or Sirri *et al.* (2010) in similar conditions. The expected FCR of 1.7 indicated in the Cobb management guide (Cobb Vantress Inc., 2008) is also, considerably higher.

Water consumption can be considered as an important welfare indicator (Manning, 2007). The average weekly water consumption (mean±s.e.) was 1.14±0.30 m^3/room for LA group and 0.94±0.26 m^3/room for C group. Therefore, total water consumption at the end of the cycle was higher in the LA group (6.8 m^3/room; 8,500 ml/bird) than in the C group (5.7 m^3/room; 7,120 ml/bird), which could be in accordance with the higher feed consumption of this group. However, water:food rate was also higher in LA group (1.96 and 2.26 for C and LA groups, respectively). These values could be considered within the normal ranges recommended by Cobb Vantress (2008) and by the poultry farming guide of best available techniques (MARM, 2010). Weekly evolution of average daily water consumption can be observed in figure 13.

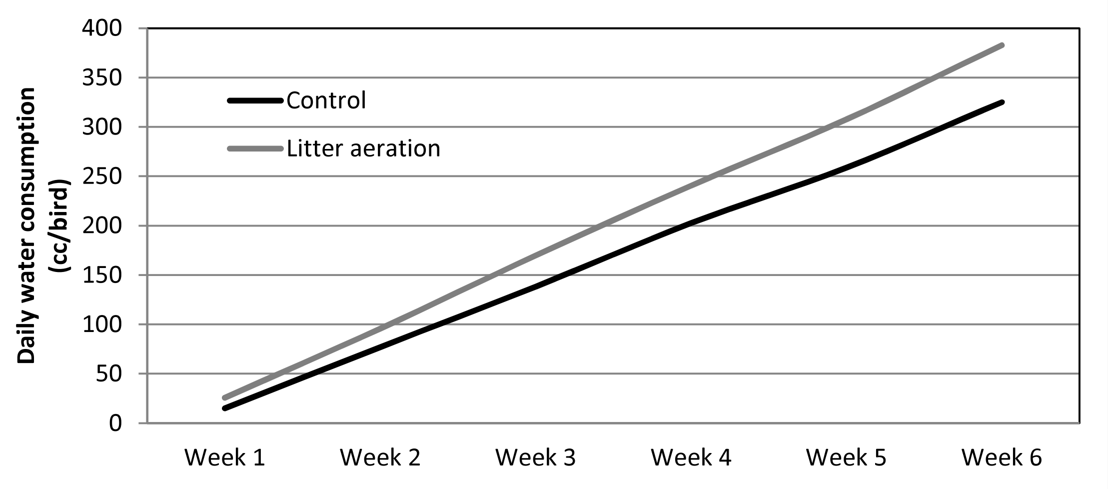

Figure 13: Evolution of average daily water consumption per bird, during the rearing cycle for both treatments

As can be observed in figure 14, at the end of the cycle, mortality rate was higher in LA group (4.5% and 5.0% for C and LA groups, respectively). However, no significant differences in mortality between treatments were found in this experiment.

These values were higher than those proposed by the European Commission in the Directive 2007/43 EC which recommends that cumulative daily mortality rate should be lower than 1% +0.06% multiplied by the slaughter age of the flock in days. These results also showed higher percentages, than those observed by Calvet *et al.* (2004), Meluzzi *et al.* (2008) or Sirri *et al.* (2010) in similar rearing conditions.

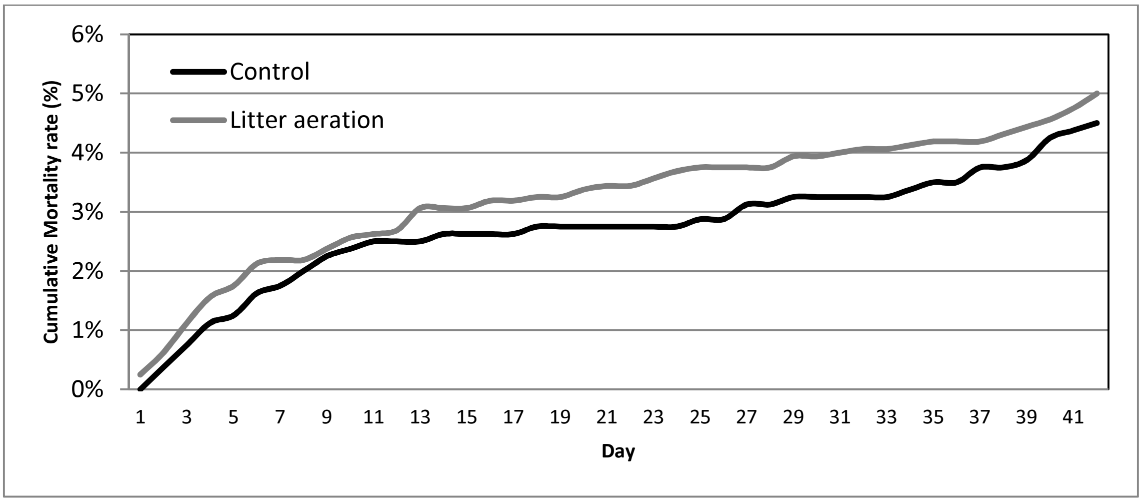

Figure 14: Cumulative mortality rate during the rearing cycle for each treatment.

Regarding the prevalence of injuries of animals, no lesions were found in chicken breasts in any of the rooms throughout the experiment. FPD CJ and HB prevalence is shown in the graphs of figure 15

Foot pads lesions were minor (most of them classified as type 1), appearing only in the last week of the study, with a prevalence of 2% and 1% for C and LA group, respectively. These results are much lower than those presented by Pagazaurtundúa and Warris (2006, 2008) and Meluzzi et al. (2008). The results could be compared with those obtained by Martland (1985) under dry litter conditions. For wet litter conditions –in the same experiment–a much higher prevalence was observed.

As it can be observed in figure 15, the percentage of animals with HB lesions was similar in both treatments but significant differences between them were found at week 4 (p=0.029). Lesions were mild in both treatments, with the vast majority of the animals classified as score 1. As in the case of FPD, the results obtained for HB could be compared with those of Martland (1985) under dry litter conditions or with the different results obtained by Meluzzi et al. (2008) with diverse bedding materials.

In the case of CJ, the percentage of affected animals was constantly higher in the C group but no statistical significance was revealed for any week.

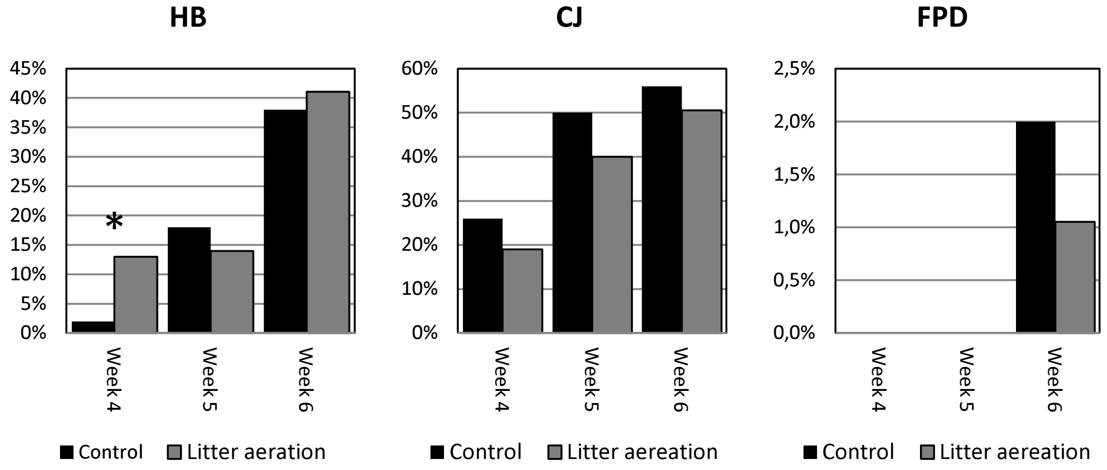

Figure 15: Prevalence of HB, FPD and CJ for each treatment, in weeks 4, 5 and 6 of the cycle

According to Berg (2004), contact dermatitis is an indicator of litter quality. Martland (1985) and Meluzzi *et al*. (2008) also reported a positive relationship between moisture of the litter and breast and feet lesions on broilers. These afirmations might agree with the results of this experiment since little differences, both in the prevalence of these pathologies and in litter characteristics, were observed.

In addition, it can be noted that the percentage of animals affected by these lesions increased with time regardless of the treatment, so it seems reasonable to assign this increment to the age of the animals as proposed by Beker *et al*. (2004).

As regards TI, it was necessary to apply a natural log transformation in order to transform the data into a normal distribution. Means (±s.e.) showed no statistical differences in TI duration between treatments (C group: 224±1 sec. and LA group: 228±1 sec; p=0.9017).

The average amount (mean ±s.e) of attempts needed to induce TI was 1.33±0.10 for the C group and 1.78±0.05. The Chi-square test, did not detect significant differences between groups (p=0.1453).

There was not statistical significance either in the difference of body temperature after the TI induction. LA group increased body temperature in 0.024±0.020 ºC whereas C group increased the temperature 0.081±0.025 ºC (mean ± s.e.).

With regard to the different parameters assessed, at the 42nd day, on the 40 culled birds (results shown in figure 16), ascites was not detected in any culled bird. No pathologies neither statistical differences between treatments were detected for heart weight (LA group=17.79±0.33 g.; C group=18.21±0.44 g.; mean ± s.e.).

Finally, no differences were either observed in the prevalence of other pathologies, except in the case of TD where LA group seems to present a higher prevalence of birds affected by this problem ($p=0.0421$) although most of these animals presented minor injuries, classified as type 1 in the majority of the cases. So, it is difficult to assign these results to a matter of welfare.

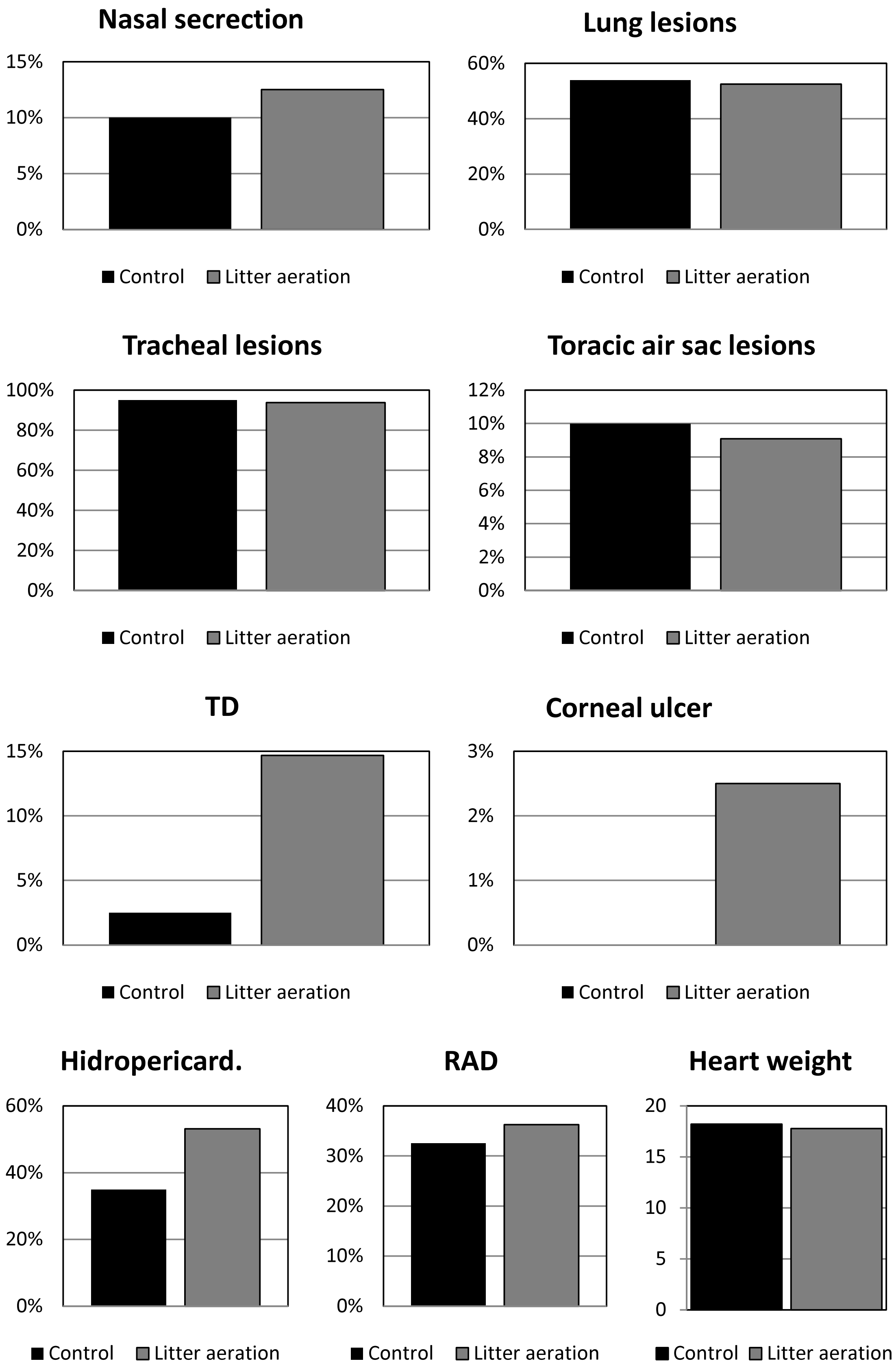

Figure 16: Prevalence of nasal secretions, lung, trachea and air sacs lesions; corneal ulcer, TD, hidropericardium and hypertrophy or dilatation of the right atrium of the heart (RAD).

4. CONCLUSIONS

According to the results observed in this experiment, it can be said that:

1. Litter aeration presented a slight effect on litter characteristics. Although pH, ash and TKN content presented higher values in litter samples from LA rooms, no statistically significant differences between treatments were found. Moreover, this technique showed little effect on its main objective: reducing litter moisture.

2. Environmental ammonia and PM concentrations, presented different patterns between treatments. Average ammonia levels were higher in the LA rooms, mainly at the end of the cycle, but they were below the values that have been proven harmful to the animals. PM concentrations were also higher for LA group and exceeded the limits recommended for human and animal health, especially after LA procedures.

3. Animal welfare seems to be affected when productive parameters are considered. Despite differences were not statistically different, birds from LA group showed a lower growth rate and a higher feed consumption once LA procedures started. Moreover, mortality rate was 0.5 percentage points higher in LA group.

4. Regarding to animal health, little differences were revealed in welfare indicators but in the prevalence of TD. The severity of this pathology was mild and the lesions were normally scored lesser than 1. No significant differences were found for other lesions prevalence neither their severity.

Consequently, in general terms, it still cannot be affirmed that LA compromises broilers performance and welfare or affects litter quality. It is needed to develop deeper analyses to broaden our understanding of this technique, both in experimental and commercial farm conditions. It is also needed to evaluate the specific effects of this technique on animals during the time of LA.

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