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Monitoring hygienic measures for decreasing *Salmonella* occurrence in Turkey Barns

Überwachung der Hygienemaßnahmen zur Reduktion des Vorkommens von Salmonella auf Putenfarmen

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Summary

The objective of this study was to evaluate a series of measures aimed at reducing the occurrence of *Salmonella* in turkeys bred in Spain, during all the stages prior to the slaughterhouse stage.

The following measures were evaluated: (a) control in feed production; B) cleaning and disinfection of farms (by thermo-fogging or spraying, using three disinfectants); c) treatment of litter using $Ca(OH)_2$; d) treatment of drinking water with an acidifying mixture based on formic acid, orthophosphoric acid and propionic acid; and (e) cleaning and disinfection of boxes, cages and vehicles.

All the measures tested proved to be effective, especially the control of feed production and the treatments for cleaning and disinfecting farms, boxes, cages and vehicles, which resulted in the absence of *Salmonella* in all the samples tested. In the case of disinfectants 3 and 4, the results were not statistically significant. Treatment of the litter reduced the percentage presence of *Salmonella* by almost 50% on untreated rearing farms, and on fattening farms by 17.5%. In terms of the treatment of drinking water, the treatment carried out in both rearing and fattening farms decreased the occurrence of *Salmonella* (34.5% and 36.2%, respectively) with respect to the percentages obtained on farms prior to treatment.

Keywords: *Salmonella,* turkey production, hygienic measures, VIDAS system

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Introduction

In the European Union (EU), and concretely in Spain, specific regulations have been developed for the control of food-borne zoonoses (European Parliament and European Council, 2003; MAPA, 2005). In breeding and fattening turkeys, the focus has mainly been on the control of *Salmonella* spp. (European Commission, 2012; MAPAMA, 2016). Reducing the occurrence of this microorganism in all stages of production is a priority objective (MAPAMA, 2016). High prevalence rates can only be combatted through a holistic view of the entire process, including feed production for the breeding and fattening of these animals.

The essential measures for controlling *Salmonella* spp. include the control of raw materials, cleaning and disinfection of equipment, warehouses and means of transport, and the thermal treatment and/or addition of authorized additives in the feed production process (MAPAMA, 2016). A European regulation also makes it mandatory for feed producers to ensure that production, processing and distribution comply with best practices, in order to guarantee feed hygiene (European Parliament and Council, 2005).

The entry of microorganisms in facilities can be avoided by controlling the raw materials used to manufacture feed and by applying biosecurity measures (MAPA, 2005). Microorganisms in feed can be destroyed by applying thermal and chemical treatments or a combination of both. Processes such as granulation, agglomeration, expansion and extrusion can be performed to sanitize feed (Ricke, 2005; Jones, 2011).

The growth of pathogenic microorganisms must be controlled on farms by applying different measures (Byrd et al., 2001; Dai Pra et al., 2009; Iba and Berchieri, 1995; Ivanov, 2001; Mueller-Doblies et al., 2014; Wolfenden et al., 2007). Fundamentally, good hygienic practices must be considered: proper cleaning and disinfection (Mueller-Doblies et al., 2014), as well as rodent control of production buildings (MAPAMA, 2016). Turkey houses can be disinfected by contact or by airborne disinfection. Some of the disinfectants most commonly used and recommended in animal health can be enhanced through combination with glutaraldehyde (Kahrs, 1995). Also, correct litter management is a critical aspect of poultry farming. Litter quality per se influences *Salmonella* populations (Santos et al., 2005). Turkey litters can mainly be treated with chemicals, notably aluminum, calcium and iron-containing compounds (Nahm, 2005), which are mixed with the litter during the breeding process. Calcium-containing compounds such as limestone may be attractive for utilization due to their low cost (Nahm, 2005). Treatments with organic acid have also been reported (Ivanov, 2001).

As regards drinking water, there is no regulation on the parameters to be controlled and their acceptable limits. The quality standards established in Spanish regulations regarding drinking water for human consumption (Ministerio de la Presidencia, 2003), must guarantee the absence of *Salmonella* spp.; the supply of acidified drinking water can contribute to this purpose (Smyser and Snoeyenbos 1979; Avila et al., 2003; Mikolajcyk, 2015; Milbradt et al., 2017), and even improve turkey performance (Cornelison et al., 2005).

In terms of the transport of animals and their equipment, national regulations establish specific procedures, as is the case in Spain (Ministerio de la Presidencia, 2005).

The objective of this study was to evaluate a series of measures aimed at reducing the occurrence of *Salmonella* in turkeys bred on Spanish farms under production conditions during all the stages prior to the slaughterhouse stage.

Materials and Methods

Feed production and control

The feed for turkey breeding and fattening was produced at three factories in Spain. Table 1 shows the biological hazard control points that were considered and the measures studied. During the reception of raw materials and premixes, a sample was collected for the laboratory and an entry record was completed for each type of raw material and premix, including all the necessary data for control and traceability. The reference values for controlling the moisture of the materials and premixes were as follows: 12.5% for soybean 47, 13% for barley, 14% for domestic wheat and 14.5% for imported wheat. The reference values for specific weight were 64 and 72kg/hl for barley and wheat, respectively. Any substances and premixes that did not comply with the aforementioned values were rejected and notified to the supplier. The received and accepted raw material was identified and stored in silos in conditions similar to those used in the normal production of the company, using the FIFO (first in, first out) method. The storage silos and circuits were made of materials suitable for their intended purpose, were independent, and were cleaned periodically (every six months). Crushing was performed using vertical hammer mills, which were cleaned daily. Dosing was performed from cells using extraction apparatus such as oscillating scrapers or augers, which discharged their contents onto the dosing scale. The ingredients were mixed in a blender for approximately 4 minutes. The mixer was cleaned daily. The liquid ingredients were incorporated into the mixer in such a way that they fell on the solid ingredients, rather than on the inner wall of the mixer. Granulation was performed mechanically and was divided into three phases: hydrothermal conditioning, compression-extrusion and cooling-drying. Hydrothermal conditioning was performed by injecting steam into a homogenizer or conditioner directly on the milled mixture, the feed reaching a temperature of 75–80 °C. Compression-extrusion was performed using granulators, containing a vertical matrix with meal compression rollers. In this stage the temperature conditions (>65°C) to which each type of production batch was subjected were controlled continuously in order to prevent pathogen survival. Cooling-drying was carried out in cooling equipment to reduce granule temperature and moisture, by means of air. The granules entered the cooler with a moisture content of 14–15% and a temperature of 60–70°C, and left with a moisture content of 11–12% and a temperature of 20–30°C. The feed was then stored (<72 hours) and shipped in bulk.

Once the feed had been produced, the presence of *Salmonella* in the feed (pellets and crumbs) produced in each of the 3 factories was monitored. For this purpose, a sample of each type of feed produced at the factories was taken once a week for one year. The sampling point was established in the storage silo prior to shipment. One hundred and fifty analyses of granular feed and 144 analyses of crumb feed were performed.

Cleaning and disinfection of farms

Disinfection was carried out in two different ways, depending on the turkey breeding stage, i.e. rearing (1–28

days) or fattening (29–105 days for hens and 29–125 days for males). On rearing farms, it was performed by thermo-fogging, after cleaning, and with a safety period of 24–36h. On the fattening farms, however, disinfection was carried out by spraying from a tank containing the diluted disinfectant. The period without turkeys was 12d, and the units tested were different to others checked for the other hygienic measures, or delayed until one month after litter treatment application in each case. A disinfectant containing didecyl dimethyl ammonium chloride, benzalkonium chloride, glutaraldehyde and isopropanol (disinfectant 1) was used for disinfection by means of thermo-fogging (Virocid®; Cid Lines NV/SA, Belgium). The dose was 1% of mixing using the Ultra Low Volume (UBV) technique for nebulization. In spray disinfection, different disinfectants were rotated to avoid the development of resistance by microorganisms. The disinfectants used, diluted to 0.5–1%, and the order of rotation, were as follows: disinfectant 2 (with glutaraldehyde, alkyl benzyl dimethyl ammonium chloride, propan-2-ol and methanol); disinfectant 3 (with hydrogen peroxide, peracetic acid and acetic acid); and disinfectant 4 (with didecyldimethylammonium chloride and glutaraldehyde). A disinfectant formulated in spray, composed of glutaraldehyde and dimethylbenzylammonium chloride, was applied for disinfection of the silos. An insecticide was used for desinsectation, the active substance of which was cypermethrin.

In order to control the efficiency of the cleaning and disinfection of the farms, 10 samples were taken randomly using sterile wipes (BioMèrieux, ref. CHI100N) at several points in the turkey houses (from different surfaces and equipment, such as walls, ventilation, troughs, water dispensers, as well as critical points). Sampling was performed before and immediately after the application of the cleaning and disinfection protocol. Sampling was repeated 7/8 times (depending on the production cycle of the barns tested) on the rearing farms and 3/4 times on the fattening farms. On the fattening farms, a sample was taken of each disinfectant used to determine whether any disinfectant was more effective than the others.

Litter treatment

The measure studied in both the rearing and fattening stages was the application of $Ca(OH)_2$, which was spread over the floor (consisting of 10cm-thick concrete, using concrete HA – 25kg/cm^2) of the turkey house using a spreader or by hand. The litter was then placed on top of it. During the time that the litter remained in the turkey house, it was moved to aerate it and prevent it from becoming too moist, which could lead to caking of the wood shavings. After the animals left the farm, the litter was removed and new litter for the next production batch was dispensed. In order to determine the impact of this measure on the presence of *Salmonella,* samples were taken using boot swabs (Stéri-Sox®; Sodibox, France) on the farms before and after the treatment. Samples were collected by placing boot swabs on the boots worn by the person taking the sample, who then walked through the different sections of the turkey house, in accordance with MAPAMA (former Spanish Ministry of Agriculture, Fisheries and Food) (2016). For this purpose, the boot swabs had to be pre-wetted with a solution containing 0.8% NaCl and 0.1% peptone. Once the samples had been taken, the boot swabs were removed, thus preventing the detachment of the adhered material. In the case of rearing farms, the boot swabs were collected just before the animals left for the fattening farms. On the fattening farms, sampling was performed 3 weeks before the fattening phase was completed.

Treatment of drinking water

The treatment consisted of adding 0.02–0.07% of an acidifying mixture (50% formic acid, 10% orthophosphoric acid and 5% propionic acid) to drinking water during the rearing and fattening steps, until pH=5.3. pH was periodically controlled using a pH-meter CRISON pH25. In order to determine the impact of this measure on the presence of *Salmonella* in barns, samples were taken using boot swabs following the procedure explained for the evaluation of litter treatment. Samples were taken from both the rearing and fattening farms. Sampling was performed in accordance with the Spanish Programme for the monitoring and control of certain serotypes of *Salmonella* in turkeys (MAPAMA, 2016; MAPAMA, 2019). To avoid confusing results, different units of farms were controlled in the application of litter treatment and drinking water measures.

Cleaning and disinfection of boxes, cages and vehicles

The boxes used to transport the animals between the different stages were cleaned and disinfected in a washing area designated specifically for this purpose, consisting of 4 well-differentiated areas: an area for storing dirty boxes, followed by an area for washing boxes, an area for storing clean boxes and, finally, an area for washing lorries. For box washing, cleaning and disinfection was carried out in automatic washing machines, at a temperature of 50ºC, dosing a non-foaming degreasing product prepared from surfactants and sequestrants in 4% solution in water. The cleaning and disinfection of the empty cages was carried out in a washing machine located on the aforementioned bay specifically for that purpose. The slaughterhouse premises had a special washing area where vehicles were cleaned and disinfected after unloading. The flow was adequate to prevent any cross contamination. The samples were taken from both boxes in the box washing area and cages in the live animal loading bay. In the case of boxes and cages, a sample was taken per box/cage, with 10 samples taken daily on 10 different sampling days. For sampling, sterile wipes (bioMérieux, ref. CHI100N) were used.

Determination of *Salmonella*

For this purpose, the VIDAS™UP SALMONELLA protocol (BioMèrieux, Madrid, Spain), an automated qualitative test for *Salmonella* based on phage recombinant protein technology, was used in accordance with ISO 16140. For the preparation and analysis of the samples, 1:10 dilutions of samples in peptone water were prepared into sterile bags using an automated gravimetric diluent. The selective enrichment supplement was then added for *Salmonella,* at a rate of 2 ml per 25 g of sample to be analyzed. The contents of the sample were homogenized in a Stomacher™ and then incubated for 18–24 h, at 41.5 ± 1°C. After the incubation phase, a subculture of 1 ml of sample was prepared in 9 ml of selective enrichment broth for *Salmonella* (SX2 broth). These tubes were incubated again at 41.5 ± 1°C for 6–24 h. After incubation, 500µl of sample was added to a *Salmonella* detection cartridge and heated for 5 ± 1 min by dry heat (VIDAS® Heat&Go, BioMèrieux, Madrid, Spain). After heating, the samples were placed in the immuno-analyzer, where all the steps of the analysis were automatically performed after the VI-

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TABLE 1: *Control points for the biological hazards considered and measures studied.*

Feed production phase	Hygiene measures
Purchase and reception of raw materials	Organoleptic control Humidity control Specific weight control Mixture of acids at the entrance
Storage of raw materials	Use of the FIFO Method Cleaning
Grinding	Cleaning of the mill
Weighing and dosage	Cleaning of equipment
Mixing	Cleaning of equipment Contaminant control (laboratory analysis)
Granulation and cooling	Hydrothermal conditioning temperature control Compression-extrusion temperature control Cooling-drying temperature control Cooling-drying humidity control
Storage and shipping	Control of storage time Vehicle cleaning
Biosafety of facilities	Design of facilities Vehicle cleaning

DAS®SPT solution had been selected. The result was obtained in 48 min, and was expressed as presence/absence of *Salmonella*.

Statistical Analysis

Statistical analysis was carried out using the SPSS 15.0 Software package (IBM Company, USA). The efficiency of the tested measures was determined by comparing the percentage of *Salmonella* occurrence before and after the treatment. For this purpose, a non-parametric chi square (X^2) test with a level of significance of 5% ($P < 0.05$) was performed. To confirm the efficacy of the disinfectants studied, the Fisher exact test ($P < 0.05$) was used by comparing results before and after the application of each disinfectant.

Results

Feed production and control measures, as well as those applied for the cleaning and disinfection of boxes, cages and vehicles, proved to be effective as shown by the absence of *Salmonella* after their application. The results of the cleaning and disinfection measures applied on the farms are shown in table 2. The litter treatment results are shown in table 3. The treatment of drinking results are shown in table 4.

Discussion

Animal feed may serve as a carrier for a wide variety of microorganisms (Maciorowski et al., 2007). Microorganisms can affect feed quality negatively by reducing dry matter and nutrients, causing musty or sour odours, and caking of the feed and/or producing toxins, as well as by carrying animal and human pathogens (Maciorowski et al., 2007). Different authors have reported the occurrence of *Salmonella* in both crumb and pelletized feed (Bucher et al., 2007; Cox et al., 1983; Jones, 2011; Jones and Richardson, 2004). The results obtained in the present study, revealing the absence of *Salmonella,* indicate that the action protocol should be maintained due to its effectiveness. The result in crumb feed was striking. This feed was not subjected to the granulation process, hence the risk of contamination was greater as no heat or mechanical treatment was applied to ensure the feed produced was sterile. Storage for a short period of time minimizes the risk of contamination (Maciorowski et al., 2007). Moreover, the 65ºC limit was considered adequate for this purpose and to avoid contaminants. Microorganisms present in raw materials, such as *Salmonella,* are mostly thermosensitive and the combination of temperature and pressure applied during the granulation process, as well as the time during which they are applied, together with humidity, are the main factors for reducing the occurrence of microorganisms in feed (Stott et al., 1975; Maciorowski et al., 2004). Thus, the feed obtained must be practically sterile. This was confirmed in our experiment.

The cleaning and disinfection results obtained for the 4 disinfectants used were highly satisfactory, although the differences between the occurrence of *Salmonella* before and after the treatment cannot be considered statistically significant in the case of disinfectants 3 and 4 (P=0,14 and P=0.054, respectively), probably due to the low number of positive cases prior to the treatment. Starting with initial percentages of between 15.4% and 44.4% of the farms affected, the absence of *Salmonella* was achieved on those farms when both thermo-fogging and spray treatments were applied. In the case of thermo-fogging treatment, quaternary ammonium compounds dissolve the cell membrane of the bacteria and the virus protective capsule, and this action is enhanced by isopropanol (Marques Ribeiro et al., 2015). Other compounds, such as glutaraldehyde, block the action of enzymes and denature proteins. The effectiveness of glutaraldehyde against *Salmonella* has been studied previously (Carrique-Mas et al., 2009). According to Mueller-Doblies et al. (2014), the total number of positive samples found at a post-cleaning and disinfection visit is correlated with the probability of carry-over of infection, whereas the location of the positive samples seems to be less important.

The treatment of the litter induced alkalinization (pH>8.5), thus preventing the development of many bacteria. In fact, the addition of 300 g/m² of lime to the floor of the broiler breeding houses increased pH approximately

TABLE 2: *Detection of Salmonella before and after cleaning and disinfection of farms.*

	Sampling timing	No. of farms sampled	*Salmonella*-positive (%)
Rearing farms (disinfection by thermo-fogging)			
Disinfectant 1 (sampling 1)	Before C&D[a]	20	7 (35)
	After C&D	20	0 (0)
Disinfectant 1 (sampling 2)	Before C&D	20	6 (30)
	After C&D	20	0 (0)
Disinfectant 1 (sampling 3)	Before C&D	20	4 (20)
	After C&D	20	0 (0)
Fattening farms (disinfection by spray)			
Disinfectant 2	Before C&D	9	4 (44.4)
	After C&D	9	0 (0)
Disinfectant 3	Before C&D	13	2 (15.4)
	After C&D	13	0 (0)
Disinfectant 4	Before C&D	8	3 (37.5)
	After C&D	8	0 (0)

[a] C&D: Cleaning and disinfection

TABLE 3: *Influence of litter treatment on the presence of Salmonella.*

Time of sampling	Farms	Samples	*Salmonella*-positive samples (%)
Rearing farms			
Before $Ca(OH)_2$ added	20	160	32 (20.00)
After $Ca(OH)_2$ added	20	164	18 (10.98)

[a] Each farm was sampled 7 or 8 times, depending on the number of cycles of production by year

Fattening farms			
Before $Ca(OH)_2$ added	322	1117	275 (24.62)
After $Ca(OH)_2$ added	322	1206	245 (20.32)

[a] Each farm was sampled 3 or 4 times, depending on the number of cycles of production by year

TABLE 4: *Detection of Salmonella before and after the acidification of drinking water.*

Time of sampling	Farms	Samples	*Salmonella*-positive samples (%)
Rearing farms			
Before acidification	20	160	32 (20)
After acidification	20	405	53 (13.1)
Fattening farms			
Before acidification	322	1117	275 (24.6)
After acidification	322	1506	236 (15.7)

ten-fold and reduced the number of CFUs of *Salmonella* by over 80%. At higher concentrations of quicklime, a reduction in the total colonies of this pathogen present in the litter can be achieved (Dai Pra et al., 2009). In addition to creating a hostile environment for microorganisms due to the variation in pH, lime reduces litter water activity. In our experiment, the results obtained after the treatment of the litter significantly reduced ($P<0.05$) the percentage of farms affected by *Salmonella* in all the cases (Tab. 2).

In relation to the treatment of drinking water, the aim was to reduce pH to 5–5.5. Additionally, the formation of biofilms inside water conduits is prevented and intestinal pH is also reduced (Ávila et al., 2003). This creates an inadequate environment for the development of pathogenic microorganisms, such as *Salmonella.* In our experiment, the treatment applied in both rearing and fattening farms resulted in a significant ($P<0.05$) decrease (34.5% and 36.2%, respectively) with respect to the percentages obtained on the farms prior to treatment, as can be seen in table 3. Byrd et al. (2001) evaluated the use of selected organic acids (0.5% acetic, lactic, or formic) in drinking water during a simulated 8-h pre-transport feed withdrawal. Treatment with lactic acid (31/100) of *Salmonella Typhimurium* recovery or formic acid (28/76) caused a significant reduction in incidence compared with 53/100 control crops acid-treated broilers. Wolfenden et al. (2007) also reported a decrease in *Salmonella enteritidis* recovery using a mix of organic acids in drinking water, and even improved the effect by including probiotics. Iba and Berchieri (1995) also reported a strong antibacterial effect of a formic acid-propionic acid commercial mixture against different *Salmonella* serotypes, but in this case mixing the acid product in feed, not in drinking water.

Finally, the cleaning and disinfection treatment applied to boxes, cages and vehicles resulted in the complete absence of *Salmonella.*

In conclusion, all the measures tested proved to be effective given the objectives of the study, prior to the slaughterhouse step.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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