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Evaluation of Hazards in a Broiler Farm

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Abstract: In order to develop a model of food quality in practical form it is necessary to implement preventive hygienic measures tending to supply the reliability and safety of the products they consume. Good Manufacture Practice (GMP), Sanitation Standard Operating Procedures (SSOP), and Hazard Analysis and Critical Control Point (HACCP) have increased in importance together with the preventive control of food. Study of hazards was developed for poultry farm with the purpose of avoiding infections by different pathogens. During the development of the breeding step diagram for broiler chicken in the poultry farm, we detected some hazards that could affect the final product quality. Winter samples showed that litter moisture in the starter house was significantly higher than humidity determination in samples obtained in the summer. Growing house litters, filamentous fungi and yeasts were isolated. The presence of mycotoxins (aflatoxins and zearalenone) was not detected in samples of corn grain and balanced food despite the high percentage of humidity in both foods. As a preventive measure against microbial infections, we recommended that all balanced feeds administered throughout the entire growing stage should be supplemented with probiotic strains. The results of this study will be beneficial to the food industry in designing strategies to effectively eliminate different pathogens in the meat products used, and our findings stress the need for increased implementation of hazard analysis and consumer food safety educational efforts.

Key word: Hazards, broiler, farm

Introduction

The Poultry Industry has become a highly competitive sector, with increasingly tight profit margins. Within the framework of the planet's globalization, which entails easy access to new technologies and knowledge and in a complicated regional context because the exchange rate imbalance and structural inequality with other countries, the efficiency and production quality improvement is a must.

Chickens, turkeys, ducks and geese, have an important role in the economy of several countries where their production is becoming increasingly organized, specialized and oriented to be industries of greater national and international relevance.

Biosecurity is a set of practices that limit the spread of disease-causing organisms. When teamed with disinfection and sanitation procedures, biosecurity practices can eradicate or reduce pathogens to noninfectious levels. Such preventive measures as vaccination and serologic monitoring also help insure good flock health.

Inadequate biosecurity can contribute to industry wide epidemics of highly pathogenic or exotic disease, resulting in quarantine and possible condemnation of flocks. An infection by a virulent organism can be just as devastating economically, reducing production over the life of the facility without overt signs of disease. Once contaminated with pathogens, poultry facilities are extremely difficult and expensive to clean, sanitize and disinfect.

The concept of biosecurity is not exclusive of poultry farming. It has been adopted worldwide and has to be adapted from general rules. The starting point could be summarized in the implementation of preventive hygienic measures tending to supply the reliability and safety of the products they consume (National Advisory Committee in Microbial Criteria for Foods, 1992). In recent years, Good Manufacture Practice (GMP), Sanitation Standard Operating Procedures (SSOP), and Hazard Analysis and Critical Control Point (HACCP) have increased in importance together with the preventive control of food (Bryant *et al.*, 2003 and Ropkins *et al.*, 2003,).

It is essential to know and understand the meaning of HAZARD, "a biological, chemical or physical quality that may make a certain food unsafe for consumption". Although the consumer tends to consider that chemical dangers are the most important ones, the biological hazards pose the greatest problem to consumers in the short run on account of their capacity to produce Foodborne Diseases (Mortimore and Wallace, 1995).

Usually, certain animal and human infections are treated with antimicrobial substances, antibiotics. One of the undesirable effects of this treatment is the proliferation of resistant strains as a consequence of their indiscriminate use. Frequently chickens, especially those that have been fed with balanced diets supplemented with antibiotics, excrete strains of

resistant *Salmonella* that may produce cross-contamination during slaughter (Arvanitidou *et al.*, 1998; Mandal, 1991 and Wray *et al.*, 1993). Food contamination with pathogens can occur at multiple steps along the food chain, including production, processing, distribution, retail marketing, and handling or preparation. This supports the need to find an alternative preventive method.

Health, management, genetics and feeding are the basements for the poultry industry. Educating those that are closely and permanently in contact with the fowls and verifying whether they comply with every measure affecting isolation, cleanliness and disinfection guarantees the success of every biosecurity plan. These measures, which may seem obvious, are not correctly executed by many farms, even when they represent a point of inflection in the evaluation of poultry production performance.

The aim of this work was to study the hazards during chicken production stage in a mid-production poultry farm in Tucumán, Argentine, with the propose to prevent microbial infections.

Materials and Methods

Poultry company study: The study was developed for a medium size poultry company in Tucumán, Argentine. It produces 45 to 55 day-old broilers with a final weight of 2.2-3.0 kilograms, for obtaining carcasses, giblets and paws.

The poultry farm covers 4,200 square meters, has 4 broiler breeding houses (200 square meters each), 1 house for balanced feed preparation and another for a warehouse. The house orientation is southwest-northeast with an easy accessed from the road. This access and the internal way are not paved, which would allow the transmission of microorganisms that are potentially pathogenic for the birds.

Microbiologic analysis of water: Microbiologic analyses of water samples from 4 wells and from 3 different supply tanks to the broiler house were carried out using the membrane filter (MF) technique under standard procedures. Filtered 100 ml of the water samples through 0.45 μm (pore diameter) membranes and collocated them in different selective agar media: Endo (total coliforms), FC (fecal coliforms), SK (streptococci), HPC (total heterotrophic aerobes) and Cetrimide (*Pseudomonas*). The plates were incubates during 24-48 h at 35°C (Endo and Cetrimide Agar) or 37°C (FC and SK).

Determination of moisture content and fungi in feed and litter samples: One sampling was performed in Winter and the other in Summer. Random samples were taken from litters (rice bran), corn and balance food in different houses. Moisture content was determinated using the dry weight technique. Two grams of samples were dried in an oven at 120°C until constant weigh. The samples (three replicates) were weighed and the initial water content was determined (Bueno *et al.*, 2001). Total fungi count (UFC g^{-1}) of litter and balanced food were performed by serial dilutions and plated on Dichloran Rose Bengal Chloramphenicol Agar (DRBC, Difco) and Potato Dextrose Agar with Chloramphenicol (PDAC, Britania). The plates were incubated for 7 days at 28°C.

The percentage (%) of contaminated grains was studied in corn grains by sowing 9 grains threefold in 3 different media, PDAC, DRBC and Czapek-Dox agar with the addition of chloramphenicol (Gonzalez *et al.*, 1995).

Isolated fungi were identified according to the method proposed by Pitt and Hocking (1997).

Mycotoxin determination in feeds: Aflatoxins and zearalenone analyses were performed by thin-layer chromatography (TLC), following the methodology proposed by Thomas *et al.* (1975) for corn and feedstuffs. The extraction was performed with methanol-water. The extract was defatted with hexane. The aflatoxins and zearalenone were subsequently extracted into chloroform and were detected by TLC. The silica gel plates were developed in chloroform: acetone (96:4) solvent system and observed under UV light for the detection of various aflatoxins and zearalenone by comparison with a standard (Sigma Chemical Co.). Chemical confirmation of aflatoxins was done by spraying 30% H_2SO_4 on TLC plates.

Results and Discussion

An interdisciplinary team was formed of specialists in food microbiology, farm animal management, fungal diseases, poultry breeding and probiotic foods for poultry with the purpose of avoiding hazards present in the breed of chicken. In this manner, firstly, when we studied the development of the breeding step diagram for broiler chickens in the poultry farm (Fig. 1), we detected some hazards that could affect the final product quality (Table 1). Every raw material going into the farm, from feeds to chicks themselves, was bought with no suppliers´certificate, for example there were no certificates stating that chicks had been vaccinated against different diseases, i.e., Marek and Gumboro.

Drinking water was obtained from a well undergoing no periodical microbiologic controls. Every breeding house had its own water supply tank to which antibiotic agents were added.

Water that is safe for human consumption is generally considered suitable for poultry (Swick, 1998). Ground-

Table 1: Hazards identification during broiler chicken breeding

Stage	Hazards	Critical Limits	Preventive Measures
Raw Materials			
Feed	Major components[a] (Mycotoxins)	$< 20 \mu g\ kg^{-1}$ (total aflatoxin) $5 \mu g\ kg^{-1}$ (aflatoxin B_1)	Supplier certificates Analysis certificate Internal raw material quality control
	Minor components[b]		Supplier certificates Internal raw material quality control
Litter	Fungi		Reduce moisture content
Drinking Water	Microbial contamination	absence	Weekly microbiological analysis
Chicks	Diseases		Supplier certificates
	High density during transportation		Vaccination certificates
Raw Material Storing	Plagues		Rodent control, Protect deposit
	Moisture (Fungi and Mycotoxins)	Humidity: < 12%	Protect deposit
	Poor building		
Breeding stage:			
Starting	Pathogen: Microorganisms		Probiotics
Growing			Antibiotics
Finishing			Bird Controls
Transport	High density		Reduce number of chicks per box
	Transportation schedule disregarded		Transport at night or reduce light power

([a]): corn, sorghum, etc.; ([b]): low quantity of ingredients in food

Table 2: Microbiological analysis of well and houses water samples

	Normal	Well[c]	House[d]
Total coliforms[a]	0-100	3250 ± 200	18 ± 5
Fecal coliforms[a]	0-50	240 ± 30	1 ± 0,5
Fecal streptococci[a]	0	15 ± 5	0
Pseudomonas[a]	0	0	0
Total Heterotrophic Aerobes[b]		> 100	> 100

[a] (CFU 100 mL^{-1}); [b] (CFU mL^{-1})

[c]Samples were taken in Winter and Summer

[d]Samples were taken from different houses in Winter and Summer

water for drinking had never undergone physiochemical or microbiological testing for quality. Moreover, every house had an independent tank supplying water automatically to the chicks. When water was subjected to microbiological tests at different times of the year, unlike samples obtained from the different tanks, groundwater was shown not to comply with the minimum requirements for drinking (Table 2). This made us think that antibiotic substances were being added to tanks indiscriminately, which could indirectly promote the onset of infectious diseases by resistant strains.

The risk of acquiring a water-borne disease is tightly connected with the type of microorganism that is presented in the water, the infective dose, the virulence of the strain, the age and the immunologic situation of the susceptible host. Other independent factors of the agent-host relationship, such as the synergic relations with other organisms that may be present in the water or the presence of certain chemical products, may contribute to increase risk (McJunkin, 1988).

Ideally, drinking water should not include any pathogenic microorganism or bacterium that may be indicative of fecal contamination. Usually, the microbiological quality of drinking water is evaluated indirectly by fecal contamination indicators, which the total coliform group is the most important. Standards for animal drinking water indicate that there should be fewer than 100 bacteria of all types per ml of water

Table 3: Mycoflora and moisture content of poultry litter

	House	Moisture Content (%)	Fungal Count (UFC g^{-1})	Fungi
First Sampling	Starting	48.27 ± 2.66	ND	ND
	Growing	28.12 ± 4.22	1.91 10^6	*Mucor spp.*, *Penicillium spp.* yeasts
	Finishing	23.58 ± 1.01	ND	ND
Second Sampling	Starting	25.30 ± 2.31	7.2 10^7	*Aspergillus flavipes*, *Aspergillus candidus*, *Penicillium spp.*, yeasts
	Growing	29.30 ± 4.16	4.6 10^3	*Eurotium spp.*, *Emericella nidulans*, *Penicillium spp.*, yeasts
	Finishing	34.00 ± 5.29	2.14 10^4	*Mucor circinelloides*, *Scopulariopsis sp.*, Aspergillus spp., *Fusarium spp.*, *Syncephalastrum racemosum*, *Geotrichum candidum*, *Penicillium spp.*, *Aspergillus candidus*

Table 4: Moisture content and mycoflora of poultry feed

	Moisture content (%)	Fungal Count (CFU g^{-1})	Infection Percentage (%)	Fungi
Balanced Diet	18.89	2.6 10^5	--	*Fusarium spp.* *Penicillium spp.* *Mucor spp.* Yeasts
Corn Grain	18.03	ND	100	*Fusarium spp.* *Penicillium spp.* *Mucor spp.* *Rhizopus spp.* yeasts *Mycelia sterilia*

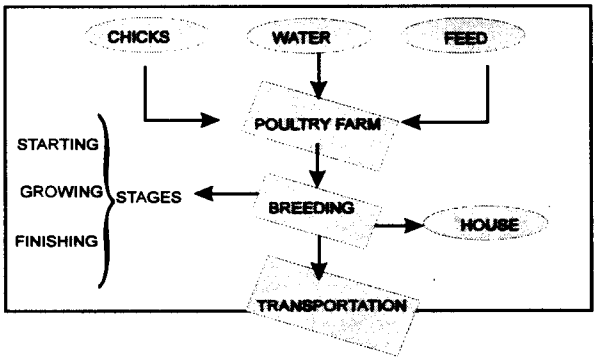

Fig. 1: Diagram of chicks breeding stages in the poultry farm

and fewer than 50 coliform bacteria per ml. Recent field research indicates that a bacteria level zero may be desirable to obtain optimum performance (Carter and Sneed, 1998).

Total Heterotrophic Aerobes indicate the system's microbiological situation and not necessarily a potential hazard to health. However, some bacteria have recently been implicated as pathogens in drinking water. In contrast, the presence of *Pseudomonas* spp. does not always represent a risk to health (Toranzos and McFeters, 1997).

Under the Argentine Food Code (Código Alimentario Argentino, 1996), potable water for public supply and for residential use is water apt for consumption and for household use. This water must not contain harmful chemicals (organic or inorganic) or microorganisms that

are detrimental to health. Article 982 states that water for consumption should not contain more than 3 UFC of total coliform bacteria by 100 ml and no *Escherichia coli* and *Pseudomona aeruginosa*.

Litter is used primarily for the purpose of keeping the bird clean and comfortable. Also, it can be used as good source of crude protein and some minerals, especially to rumiant (Martin *et al.*, 1998). It absorbs moisture from the droppings and then gives the moisture to the air brought in by ventilation (Ensminger, 1992). Generally, variations occur in litter due to bird type, feeding regimen and house management. Moisture is important due to the added weigh that high moisture will add to hauling cost and its effect on litter quality (Britton and Bullard, 1998). Proper ventilation lower the humidity, keep the litter dry and improves the oxygen interchange (Ensminger, 1992).

It is generally recommended that litter moisture can be in the 25-30% range, that is a very good percentage to permit the fungi growth. Winter samples (first sampling) showed that litter moisture in the starter house was significantly higher ($P<0.05$) than in the growing and finishing house; humidity determination in samples obtained in the summer (second sampling) did not show significant differences among houses (Table 3). Our values matched those by Martin *et al.* (1998) and were higher than Britton and Bullard (1998). In general, samples from litters that were positive for *Salmonella* showed humidity levels in excess of 21% (deGraft-Hanson *et al.*, 2000).

It is important to highlight that a high mould count was found in the winter samples from the growing houses litters, whereas summer values were significantly higher ($P<0.05$) in litters in the starting house vs. the remaining two; the highest fungi count was found in litters with lower humidity percentage (Table 3). Similar results were published by Skrinjar *et al.* (1995) and Vissiennon (1999).

In the first sampling of growing house litters, filamentous fungi and yeasts of the genera *Mucor* sp. and *Penicillium* sp. were isolated. In the second sampling, a widest variety of genera were identified: *Aspergillus*, *Eurotium* sp., *Emericella* sp., *Geotrichum sp.*, *Mucor sp.*, *Penicillium* sp., *Syncephalastrum* sp. and yeasts (Table 3). It should be noted that some isolated fungi (*Aspergillus* and *Penicillum*) are potential producers of mycotoxins. Skrinjar *et al.* (1995) describe that *Aspergillus*, *Penicillium* and *Mucor* were the most prevalent genera in samples from litters. No dermatophytes have been isolated in any of the analyzed samples, this fact coinciding with findings by Skrinjar *et al.* (1995).

In the poultry farm, diets are prepared using different ingredients whose composition varies according to the growing stage of birds, with the corn proportion being increased for older ages. Moisture analysis in feed samples of balanced diets and corn grains showed similar percentages (18%) (Table 4). Balanced diets have been shown to exhibit high fungi counts (Table 4), but between the values that Dalcero *et al.* (1997) found. In general, the quantification of fungi is a highly useful indicator to determine the health and hygiene quality of supplies. This count should not exceed 10^5 UFC g^{-1} (Luna, 1997). The contaminated grain percentage technique is used for determination of external mycoflora (Pitt and Hocking, 1997). The analyzed corn grains lack optimum quality for consumption as they showed 100 % infection. The same fungal genera were isolated and identified in the different analyzed feeds. In addition, other fungi were isolated in corn grains: *Rhizopus* sp. and *Mycelia sterilia* (Table 4). Matching the findings in litters, potentially mycotoxigenic fungi were isolated from bird feeds.

Environmental conditions must be favorable for fungal spores to germinate, grow and reproduce. The two factors having a crucial effect on fungal growth and mycotoxin production are humidity and temperature. Generally, mold growth is favored by high humidity in combination with high temperatures (Bullerman *et al.*, 1984). Most frequently, the limiting requirement for fungal development is moisture content which, in theory, should not exceed 11.50% (Wyatt, 1990). In our studies, the moisture content in the food is higher than the above value, fostering considerable fungal growth.

While poultry mycotoxicosis is caused by fungus colonization and invasion of grains and feeds, other environmental aspects are involved. Aflatoxins are highly toxic and carcinogenic fungal metabolites of *Aspergillus flavus*, *A. parasiticus* and *A. nomius*. On the other hand, zearalenone is a strogenic mycotoxin produced by *Fusarium graminearum.* Broilers and hens are more tolerant to the latter toxin than turkeys and pigs. While most data show zearalenone as being safer for chicks, recent information suggests that it may affect poultry negatively (Hoerr, 1991).

The presence of mycotoxins (aflatoxins and zearalenone) was not detected in samples of corn grain and diets despite the high percentage of humidity in both foods. It should be noted that at the time of sampling, chicks were presented with no clinical symptoms associated with mycotoxins diseases.

Although the technique has good sensitivity (2 μg Kg^{-1} for aflatoxin B_1, and 100 μg Kg^{-1} for zearalenone (Thomas *et al.*, 1975), we proposed serial samplings in order to prevent increased levels of mycotoxins in foods.

The breeding houses have holes through which birds and rodents may go, acting as disease transmission vectors. The food preparation and deposit house is

open and the presence of rodent fecal matter was observed on food bags. The poultry production must not live with rats. There are a number of reasons why the modern poultry farmer should not allow the presence of rodents: they provoke severe disorders to the surrounding human population and represent a strong zootechnical limitation in view of the growing economic losses they usually bring about as they transmit diseases to the fowl and contaminate or eat foods, destinated for poultry and human consumption. Salmonellosis, colibacillosis, coryza, pasteurellosis, mycoplasmosis, hemorrhagic enteritis, hymenolepiasis (tenia), capillariasis and ascaridiasis are diseases connected with rodents (Marin, 2000). To ensure an effective rodent control, an on-going program must be implemented.

In the broiler housing 35-day-old chicks, there were a certain number of fallen birds with swollen stiff legs, while others were presented with abdominal swelling and scanty feathers in the perianal region. We also observed a certain amount of dead birds. Chick necropsy revealed accumulation of large volumes of liquid in the abdominal cavity (ascites). One of the main reasons for this pathology in young chicks is the lack of ventilation in the house, which causes a reduction in the supply of oxygen aggravated by heart problems carrying to liquid accumulation in the abdominal cavity. Additionally, adult broiler chickens are probably more sensitive to acquire this pathology due to their higher metabolic rate and, therefore, greater oxygen consumption (Riddell, 1991).

Several interventions at the animal production stage have been proposed for the control of foodborne pathogens. For example, there has been considerable progress in possible *Salmonella* and *Campylobacter* reduction during broiler production; however, the research has not provided the certain reduction or elimination of these pathogens. Some possible interventions during animal production include: animal trace back, replacement progeny, vaccination, environment control, diet, feed/water, competitive exclusion and handling during transport. These are all possible interventions that could be considered as preventive measures on which a control could be based. However, these interventions need considerable research before they could be applied on a practical basis in a HACCP system for actual animal production. Hopefully, these and other interventions can be applied in a practical way some time for the control of foodborne pathogens. The current trend is to use probiotic foods as prevention and/or therapy in animals as well as in humans. While the meaning of probiotic was the object of a long debate, all authors agree on the direct or indirect benefits of these products on the health of humans, animals and plants. Recently, Guarner and Schaafsma (1998) have proposed a definition for probiotic foods: "living microorganisms which, when consumed in a certain amount, have effects on health that go beyond basic inherent nutrition". As a preventive measure against microbial infections, we recommended that all balanced feeds administered throughout the entire growing stage should be supplemented with probiotic bacteria. In our laboratory, lactic acid bacteria have been isolated from healthy chicks and selected for their properties (adhesion, production of antimicrobial substances, mycotoxin adsorption, etc.) (Gonzalez *et al.*, 1993; Gusils *et al.*, 1999b and Bueno, 2003). *In vitro* and *in vivo* studies (administration of the probiotic mix to batches of newborn chicks) determined the innocuousness of these strains and the preventive effect against chick infection from pathogens of the genus *Salmonella* (Gusils *et al.*, 1999a).

The results obtained in this work were presented to the owner of the studied poultry farm and we proposed carried out monitoring in several successive stages. Firstly, audit suppliers and distributors´records. Then, check proposed hazards to see if it is operating properly without exceeding the critical limits. Finally, consumers claims could be analyzed to ensure that every hazards have been identified and controlled. These studies are the first step in the development of HACCP systems for poultry breeding.

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