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Analysis of the Presence of *Clostridium perfringens* in Feed and Raw Material Used in Poultry Production

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Abstract

Microbiological control of feeds used in industrial poultry production has been increasingly important due to the demands of the market for food safety, as well as the need to ensure better quality of the digestive system of the birds. Microbiological analysis carried out in raw material used in feed production, especially feather, meat, and organ meal, has shown contamination by *Clostridium perfringens*. In order to study the presence of *Clostridium perfringens*, a total of 354 samples of feed and raw material were analyzed from January 2011 to July 2013. Samples came from four companies located in the state of São Paulo, with a total of 166 samples of meat meal, 24 samples of feather meal, 43 samples of organ meal, and 121 samples of feed. The following results were obtained: 88 (53%), 15 (62.5%), 16 (37.21%), and 23 (19%) samples were positive for *Clostridium perfringens* in each group of samples, respectively, with counts ranging from 2.0×10^2 to 7.0×10^3 CFU/g.

Keywords

Necrotic Enteritis; *Clostridium perfringens*; Feed

1. Introduction

Control of microbiological contamination in feed and raw materials begins with good sampling programs and laboratory analyses in order to identify sources of contamination and to determine control strategies.

Among the different vehicles of pathogens in the poultry production chain, the contamination of feed and raw material for feed production should be highlighted. These are common vehicles of different pathogenic bacteria that may cause clinical and/or subclinical effects in birds, leading to inadequate performance and, consequently,

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to economic losses. *Clostridium perfringens* is among the bacteria involved in this contamination. The agent causes necrotic enteritis in birds, and may be found in the intestinal content and feces of the birds, as well as in dirt, contaminated food, bedding material, and other sources [1].

Microbiological analyses carried out in raw material used in feed production, mainly feather, meat, and organ meal have shown different levels of contamination by *C. perfringens*, depending on the quality of the raw material and on the hygienic procedures used during processing [2]. These sources, when contaminated, may be vehicles of important pathogens for the flocks [3] [4], leading to decreased feed efficiency and health problems, which end up in the development of diseases and considerable economic losses to this production system.

In grains, soybean meal and meals of animal origin, different microorganisms may be found. Some of them are resistant to low moisture conditions and may survive for a long periods. Contamination levels reported in the literature range from 5×10^3 to 1.6×10^8 CFU/g [5] [6]. Contamination by bacteria is frequent, with *Salmonella* spp. and *Clostridium* sp. among them. The main source of contamination is the raw material, such as grains and oily seeds, which becomes contaminated by the dust of the soil where the plants are grown, and is carried by the wind, rain, and by mechanical removal of the plants in the field [1].

In several outbreaks of necrotic enteritis, feed and bedding material are among the main sources of infection. The disease is observed as acute enterotoxaemia, in clinical or subclinical form [7], and it is caused by *C. perfringens* A and C. It is characterized by ulcerative lesions and confluent necrosis of the mucosa of the small intestines, leading to debility which develops quickly. It mainly affects young animals, two to five weeks of age, and the onset is sudden, generally associated with immunosuppression. Death ensues quickly and with high prevalence [8]. Broilers, commercial laying hens, turkeys, quails, ostriches and wild birds are susceptible, with large economic losses and problems related to animal welfare, due to the increasing percentage of mortality in the clinical cases [9].

The need of controlling the presence of microrganisms in bird feed is undeniable, mainly due to the recent changes in safety regulations for foods associated with the increased demands of the consumers and related to sanitary issues. The presence of these microorganisms in bird feed may also be considered in relation to the biosafety of the lots and the performance of the animals [10].

Therefore, to maintain a microbiological analysis program in raw materials used in feed production, such as meals of animal origin, it is necessary to monitor the contamination profile of these ingredients, and to determine minimum standards of quality. The objective of the present study was to analyze the presence of *Clostridium perfringens* in feed and raw material used in commercial poultry operations.

2. Material and Methods

A total of 354 samples were analyzed, with 166 of meat meal, 24 of feather meal, 43 of organ meal, and 121 of feed, which were collected in four companies located in the state of Sao Paulo, from January 2011 to July 2013. The methodology used in this study was performed as described by Barnes [11] and FDA [12], 1 mL of each dilution was placed in Petri dishes. They were then added of 15 mL of molten TSC (Triptose Sulfite Cycloserine Agar®, BD DIFCO, Le Pont de Claix, France) or SFP (Shahadi Ferguson Perfringens Agar®, BD DIFCO, Le Pont de Claix, France). Agar in the plates was homogenized, and plates were stored in anaerobic jars, without inversion of the plates, at 36˚C ± 1˚C for 24 hours. After that, colonies were counted in plates that had from 30 to 300 colonies.

Clostridium sp. form dark colonies due to the reduction of sulfite to sulfide, which reacts with ammonium and iron citrate, forming a black precipitate. From these plates five black colonies were chosen and added to thioglycollate broth for 24 - 48 h for gas production. After gas was produced, the following biochemical tests were carried out: fermentation (based on the fermentation of milk containing iron, producing large amounts of gas, characteristic of *Clostridium perfringens*); liquefaction of gelatin and lecithinase test. When these three biochemical tests were positive for the colonies tested, samples were considered positive for the presence of *C. perfringens*.

3. Results and Discussion

The following results were obtained in the isolation of *C. perfringens*: 88 positive samples in meat meal, corresponding to 53% of them; 15 samples positive in feather meal (62.5%); in organ meal, 16 samples were positive (37.21%), and in feed samples, 23 were positive, corresponding to 19% of them. Counts ranged from 2.0×10^2 to 7.0×10^3 CFU/g. The results are showed in **Table 1**.

Table 1. Analysis of *Clostridium perfringens* for meat meal, feather meal, organ meal and feed and limits acceptable of contamination.

Sample	*Positive/total*[a]	%	*Negative/total*[b]	%
Meat meal	88/166	53	78/166	47
Feather meal	15/24	62.5	9/24	37.5
Organ meal	16/43	37.2	27/43	62.8
Feed	23/121	19	98/121	81
	Regulament [18]			
Limit acceptable	Absence			

[a]Results are expressed as number of positive samples/total number of samples tested. [b]Results are expressed as number of negative samples/total number of samples tested.

Studies carried out all over the world prove the presence of spores in products used in animal feed production, in amounts that vary according to the raw material under analysis. Wojdat *et al*. [13] detected *C. perfringens* in 38% of the samples of ingredients used in the production of broiler feed, with the greatest indices observed in meals of animal origin, once these products presented high concentrations of protein.

In the European Union, McDevitt *et al*. [14] observed that the incidence of the clinical and subclinical forms of the disease increased, ranging from 1% to 40%, after the banning of Gram-positive additives from poultry feeds.

In a study conducted in Spain, Prió *et al*. [15] observed that the level of contamination by *Clostridium* sp. found in different ingredients for animal nutrition is widely variable.

Richardson [16] analyzed the presence of different microorganisms in feed from different feed plants, and observed that the level of contamination of the feed collected in the silo of the poultry facility was always greater than the level of contamination of the feeds collected in the plants. The study pointed out that the strategies to control microbiological contamination adopted by the feed plants do not ensure the absence or low counts of bacteria and fungi until the moment of feed is placed in the feeders in the poultry facility. Therefore, the use of strategies to control the levels of contamination of the raw materials until the final destination of the feed (the feeder) is warranted.

In a survey of 2049 samples, Richardson [17] observed that *C. perfringens* may also be isolated in different ingredients used in bird and pig feed. The levels of contamination of these samples ranged from 10 to 2000 CFU/g.

Schocken-Iturrino and Ishi [8] analyzed *C. perfringens* contamination in samples of meals of animal origin, such as organ, feather and meat, used in poultry feed production, and found counts of up to 3.2×10^4 CFU/g.

In a survey conducted in three different cities of the state of Sao Paulo, Schocken-Iturrino *et al*. [4] observed that, among 90 samples of broiler feed analyzed, 42% were contaminated by *C. perfringens*, and mean counts were 3.69×10^2 CFU/g. The high counts found in this survey were associated in Good Manufacturing Practices failures, mainly the lack of hygiene in the production and little care in the storage of the feeds.

4. Conclusion

Microbiological control of feed ingredients and poultry feed has been considered increasingly relevant due to the demands of the market for food safety all over the production chain, besides the perception of greater challenges in field condition that may lead to economic losses in bird lots.

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