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Microbiological Assessment of Broiler Compound Feed Production as Part of the Food Chain—A Case Study in a Romanian Feed Mill

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Abstract: Compound feed and the raw materials used in their production are potential vectors of microbiological contamination in the food chain. The purpose of this study was to microbiologically asses raw materials (maize, wheat, soybean meal, and sunflower meal), and broiler compound feed (starter, grower, and finisher) from a representative feed mill in Romania; the microbiological contaminants that were analyzed were yeasts and molds, *Salmonella* spp., *Escherichia coli*, and *Clostridium perfringens*. Our study occured during the years 2019 and 2020; in 2019, 191 samples of raw materials and 360 samples of compound feed were analyzed and in 2020, 143 samples of raw materials and 241 samples of compound feed were analzyed. Among the tested samples of raw materials, the mean values of the yeasts and molds for maize, wheat, soybean, and sunflower meal were 1.3×10^3, 9.5×10^2, 6.4×10^2, and 7.4×10^2 cfu/g in 2019 and 1.5×10^3, 1.0×10^3, 5.2×10^2, and 7.1×10^2 cfu/g in 2020. In the analyzed compound feed samples, the mean amounts for the starter, grower, and finisher were 5.9×10^2, 4.2×10^2, and 4.2×10^2 cfu/g in 2019 and 5.3×10^2, 6.5×10^2, and 5.8×10^2 cfu/g in 2020. Potentially toxigenic fungi from *Aspergillus*, *Penicillium*, and *Fusarium* genera have been identified as the most common in all of the samples. In the raw materials, in both years the highest numbers of *Aspergillus*-positive samples were recorded: 66.6% in 2019 and 100% in 2020 for the maize samples, 50% in 2019 and 75% in 2020 for the wheat samples, 76% in 2019 and 87.5% in 2020 for the soybean meal samples and 71.4% in 2019 and 100% in 2020 for the sunflower meal. In the starter compound feed, the *Aspergillus* genera was prevailing in 2019 (46.6%), while in 2020, the species of the *Penicillium* and *Cladosporium* genera were identified in the majority of the samples (50%); for the grower and finisher compound feed, the *Aspergillus* genera was predominantly identified in 2019 (60% and 72.2% of the samples, respectively) and 2020 (61.5% and 46.6%, respectively). All of the results of the bacteriological analysis for determining the contamination with *Salmonella* spp., *E. coli*, and *Clostridium perfringens* were negative. Based on the results obtained in this study, monitoring and analysis of microbiological hazards in a feed mill will help to control and prevent contamination and have a direct impact on food safety.

Keywords: food and feed safety; yeasts and molds; *Salmonella* spp.; *Escherichia coli*; *Clostridium perfringens*

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1. Introduction

Compound feed is vulnerable to the introduction of bacteria throughout the production chain. The aim of the control of pathogens in feed is to ensure that they are under a critical threshold to minimize the risk to human and animal health [1]. The unnecessary or unintentional presence of pathogenic microorganisms is called microbiological contamination [2].

Potentially toxigenic fungi of field crops belong to the *Alternaria*, *Aspergillus*, *Cladosporium*, *Helminthosporium*, and *Fusarium* genera [3,4]. When cereal grains and feed are colonized by molds, there is a significant risk of contamination with their secondary

metabolites [5], such as mycotoxins [6–8]. In addition to impairing the nutritional value and processing, the sanitary quality of the corn kernels can chemically alter the composition of the feed through the presence of substrates produced by microorganisms [9]. A high incidence of fungi of the *Aspergillus* genus (aflatoxigenic) was identified in maize grain stored under conditions of humidity between 13–18% [4]. Zearalenone mycotoxin in high concentrations can contaminate the carcasses of broilers, implying an anabolic effect in humans [10,11].

The genus *Salmonella* corresponds to an enteric Gram-negative, facultative anaerobe and non-spore-forming bacillus with cell diameters ranging from 0.7 to 1.5 µm and lengths from 2 to 5 µm, that belongs to the *Enterobacteriaceae* family [12]. Members of the *Salmonella* genus grow under temperatures from 7 to 48 °C, tolerating growth at water activity levels up to 0.995 and pH values between 6.5 to 7.5 [13]. *Salmonella* infection in poultry has long been categorized as a zoonotic disease of economic importance in public health [14–18]. Poultry products have been considered as the major reservoir of *Salmonella*, with approximately 200 serovars isolated from them [18–22]. Broilers and broiler products are an important source of *Salmonella* [23,24] and contaminated compound feed is considered to be one of the main sources of infection with *Salmonella* [25,26]. After contaminated feed has been ingested by broilers, *Salmonella* can multiply in their gastrointestinal tract and be eliminated in feces leading to increased multiplication on the farm [27,28]. Thermal inactivation of *Salmonella* enteridis and *Escherichia coli* O157:H7 was determined by temperatures between 54.3–64.5 °C, pH values between 4.2–9.6 with HCl or NaOH, and a NaCl concentration between 0.5–8.5% [29].

Escherichia coli (*E. coli*) is a Gram-negative bacterium of the family *Enterobacteriaceae* [30]. Regularly, it is found in the small intestine of endothermic organisms. *E. coli* is not always confined to the gut, and its ability to survive for short periods outside the body makes it possible to test samples for fecal contamination [31]. Avian colibacillosis is one of the major bacterial diseases in the poultry industry that has gained substantial attention worldwide [32]. Moreover, it is important to consider the potential for its zoonotic transmission through poultry reservoirs [33].

Clostridium perfringens (*C. perfringens*) is an anaerobic spore-forming, Gram-positive bacterium capable of producing various toxins and enzymes responsible for the lesions and associated symptoms [34,35]. The colonisation of poultry with *C. perfringens* is an early incident [36,37]. As a consequence of its ubiquity, *C. perfringens* is also recovered from broiler carcasses after refrigeration [36,37]. In broilers, Clostridia can cause necrotizing enteritis, an infection of the intestinal wall that is mainly caused by *C. perfringens* type A toxin [38]; it also constitutes a risk of transmission to humans through the food chain [39,40].

The European Union's food safety policy was reformulated at the beginning of the 2000s, in accordance with the approach of an integrative concept "from farm to fork", thus guaranteeing a high level of safety for food products in all stages of the production chain (including feed production) [41]. Regulation (EC) number 2160 of 2003 [42] ensures that appropriate and effective measures are implemented for their detection and control at all relevant stages of production, processing, and distribution, including animal feed, to reduce their prevalence and the risk to public health. According to Article 5, paragraph 3 of Regulation (EC) number 183 of 2005 [43] on feed hygiene, feed manufacturers must comply with specific microbiological criteria.

Considering the inclusion of compound feed production in the food chain, in the present work, raw materials and broiler compound feed from a feed mill in Romania were microbiologically assessed; the microbiological contaminants that were analyzed were yeasts and molds, *Salmonella* spp., *E. coli*, and *Clostridium perfringens*. Our study took place during the years 2019 and 2020, and provides new data on the microbiological evaluation of compound feed production as a part of the food chain.

2. Materials and Methods

2.1. Feed Samples

Compound feed is a balanced mixture of raw feed materials in order to satisfy the energy and nutrient requirements of a species/category of animals. In Romania, the main source of energy for compound feed is cereal seeds (maize and wheat), and soybean and sunflower meal are used as protein sources [44].

The samples of raw materials used in the production of compound feed (maize grains, wheat grains, soybean meal, and sunflower meal) and compound feed for broilers in different growth phases (starter, grower, and finisher) originated from a representative feed mill in Romania in terms of production (120,000 t/year). In 2019, 191 raw material samples were analyzed (37 maize, 15 wheat, 107 soybean meal, and 32 sunflower meal) and in 2020, 143 raw material samples were analyzed (13 maize, 9 wheat, 109 soybean meal, and 12 sunflower meal). In 2019, 360 samples of compound feed were analyzed (103 starter, 90 grower, and 167 finisher) and in 2020, 241 samples were analyzed (44 starter, 84 grower, and 113 finisher). The samples of raw materials were collected from the storage silos and the samples of compound feed were collected from the bunkers for storing the finished products of the studied unit, and they transferred to the specialized laboratories.

For the raw materials analysis, elementary samples from 25 points were collected with a manual probe inserted perpendicular to the base of the silo; for the compound feed analysis, seven elementary samples from batches of 24 tons were sampled manually with a trowel. All of the elementary samples taken constituted the global sample which was divided and homogenized with the centrifugal mechanical divider and resulted in the laboratory sample.

2.2. Yeasts and Molds Analysis

Yeasts and molds were detected in according with standard SR ISO 21527-2:2009 which describes a horizontal method for the enumeration of viable osmophilic yeasts and xerophilic molds in products intended for human consumption or the feeding of animals by means of the colony count technique at 25 ± 1 °C. In order to create surface-inoculated plates, a specific selective culture medium was used. A certain amount of the initial suspension, or decimal dilutions of the suspension, was employed, depending on the anticipated number of colonies. The plates were then aerobically incubated at 25 ± 1 °C for 5 to 7 days, then the colonies were counted. The number of colonies discovered on the plates selected at dilution levels producing countable colonies was used to compute the quantity of yeasts and molds per gram of sample [45].

2.3. Salmonella spp. Analysis

Salmonella spp. was detected in according with standard SR EN ISO 6579-1:2017 which specifies a horizontal method for the detection of *Salmonella* and is applicable to the products intended for human consumption and the feeding of animals. Even when *Salmonella* is present, other *Enterobacteriaceae* or bacteria from other families are frequently present in far higher numbers. Pre-enrichment is used to enable the detection of *Salmonella* with low numbers or that have been harmed. The test portion was inoculated into buffered peptone water, and the mixture was then incubated between 34 °C and 38 °C for 18 h. The resulting culture was inoculated into Rappaport–Vassiliadis medium with soy (RVS broth) and a Muller–Kauffmann tetrathionate–novobiocin broth (MKTTn broth). The MKTTn broth was incubated at 37 °C for 24 h whereas the RVS broth was incubated at 41.5 °C. From the cultures obtained, a selective solid media was inoculated with xylose lysine deoxycholate agar (XLD agar); the XLD agar was incubated at 37 °C and examined after 24 h. *Salmonella* subcultures were used to subculture colonies, and the identities of the colonies were verified using the proper biochemical and serological tests [46].

2.4. Escherichia coli Analysis

Escherichia coli was detected in according with standard SR ISO 7251:2009 which gives general guidelines for the detection and enumeration of presumptive *Escherichia coli* by means of the liquid-medium culture technique and calculation of the most probable number (MPN) after incubation at 37 °C, then at 44 °C. The initial suspension was inoculated into three tubes of double-strength liquid selective enrichment medium in a predetermined amount. A predetermined amount of the initial suspension was inoculated into each of the three tubes of the single-strength liquid enrichment medium. A specific number of decimal dilutions of the initial suspension was then added to three more tubes of the single-strength medium under the identical conditions. After 24 and 48 h of incubation at 37 °C, the double- and single-strength medium tubes were checked to see if any gas was being produced. Each tube contained a liquid selective medium and was subcultured to a tube containing a double-strength medium that had produced opacity, cloudiness, or gaseous emissions, as well as each tube containing a single-strength medium that had produced gaseous emissions (EC broth). The obtained tubes were incubated at 44 °C for up to 48 h, and after 24 h and 48 h, they were checked for gas production. Each tube of media that had produced gaseous emissions was subcultured with a tube of peptone water free of indole. The obtained tubes were incubated at 44 °C for 48 h before being checked for indole formation as a result of tryptophan being broken down in the peptone component. The MPN table from Standard, which takes into account the number of tubes of single- and double-strength medium whose subcultures have produced gas in the EC broth and indole in the peptone water at 44 °C, was used to calculate the most likely number of presumptive *Escherichia coli* [47].

2.5. Clostridium perfringens Analysis

Clostridium perfringens was detected in according with standard SR EN ISO 7937:2005 which is applicable to products intended for human consumption and the feeding of animals. A predetermined amount of the original suspension was inoculated into Petri dishes as an inoculant. Under the same circumstances, additional Petri dishes were inoculated using decimal dilutions of the test sample or the initial suspension. An overlay of the same media was applied after adding a selective medium (poured-plate technique). The plates were anaerobically incubated at 37 °C for 20 h and two hours, and the distinctive colonies were enumerated. The quantity of distinctive colonies was verified, and it was computed as to how many *C. perfringens* bacteria there were per gram of sample [48].

2.6. Statistical Analysis

The data obtained from the analyses were processed and interpreted statistically. The minimum and maximum values were established and the position and variation estimators were calculated, respectively, the arithmetic mean ($\bar{x}$) and the standard deviation (s), for the samples that recorded positive results. The means and standard deviation were calculated using Microsoft Excel 2016 [49].

3. Results

3.1. Raw Materials

The results of the microbiological assessment (yeasts and molds, *Salmonella* spp., *E. coli*, and *Clostridium perfringens*) of the raw materials (maize, wheat, soybean meal, and sunflower meal) are presented in Table 1.

Table 1. Results of microbiological assessment of raw materials.

Specification		2019 Year						2020 Year					
		n	**Positive (%)**	**$\bar{x}$**	**s**	**Min.**	**Max.**	**n**	**Positive (%)**	**$\bar{x}$**	**s**	**Min.**	**Max.**
Yeasts and molds (cfu/g)	Maize	17	88.2	1.3×10^3	12.4×10^2	6.0×10^2	4.3×10^3	13	84.6	1.5×10^3	1.6×10^3	4×10^2	4.9×10^3
	Wheat	4	100	9.5×10^2	9.7×10^2	4.0×10^2	2.4×10^3	4	100	1.0×10^3	1.1×10^3	4×10^2	2.8×10^3
	Soybean meal	27	92.5	6.4×10^2	2.5×10^2	4.0×10^2	1.0×10^3	28	85.7	5.2×10^2	1.9×10^2	4×10^2	1.0×10^3
	Sunflower meal	7	100	7.4×10^2	2.5×10^2	4.0×10^2	1.0×10^3	3	100	7.1×10^2	3.0×10^2	4×10^2	1.0×10^3
Salmonella spp. /25 g	Maize	8	0	0	0	absent/25 g		0	-	-	-	-	-
	Wheat	4	0	0	0	absent/25 g		2	0	0	0	absent/25 g	
	Soybean meal	32	0	0	0	absent/25 g		39	0	0	0	absent/25 g	
	Sunflower meal	10	0	0	0	absent/25 g		4	0	0	0	absent/25 g	
E.coli (cfu/g)	Maize	7	0	0	0	0	0	0	-	-	-	-	-
	Wheat	3	0	0	0	0	0	1	0	0	0	0	0
	Soybean meal	31	0	0	0	0	0	39	0	0	0	0	0
	Sunflower meal	10	0	0	0	0	0	4	0	0	0	0	0
Clostridium perfringens (cfu/g)	Maize	5	0	0	0	0	0	0	-	-	-	-	-
	Wheat	4	0	0	0	0	0	2	0	0	0	0	0
	Soybean meal	17	0	0	0	0	0	3	0	0	0	0	0
	Sunflower meal	5	0	0	0	0	0	1	0	0	0	0	0

n—number of samples analyzed. $\bar{x}$—mean. s—standard deviation. Min.—minimum value identified. Max.—maximum value identified.

The mean values established for the analysis to determine the yeast and mold contamination of the maize were 1.3×10^3 cfu/g in 2019 for 88.2% of the positive samples, and a maximum value of 4.3×10^3 cfu/g and of 1.5×10^3 cfu/g in 2020 for 84.6% of the positive samples with a maximum value of 4.9×10^3 cfu/g. Compared to the number of analyses carried out to determine the content of yeasts and molds in soybean meal, a proportion of 92.5% of positive samples was identified in 2019 and 86.7% in 2020, and also the average values of 6.4×10^2 cfu/g in 2019 5.2×10^2 cfu/g in were identified in 2020.

Regarding the isolation proportion of potentially toxigenic fungi genera from the maize and wheat grains analyzed, the graphic representation (Figure 1) highlights the five genera that were identified (*Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, and *Alternaria*).

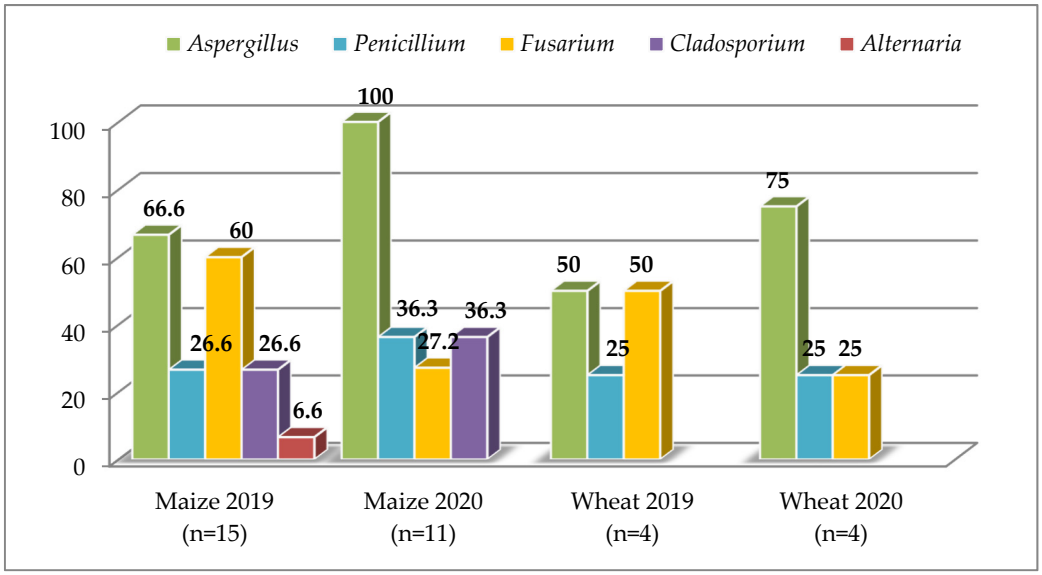

Figure 1. Proportion (%) of potentially toxigenic fungi genera in positive maize and wheat samples (n—number of positive samples).

Regarding the proportion of potentially toxigenic fungi genera identified in soybean and sunflower meal (Figure 2), five genera were identified (*Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, and *Mucor*).

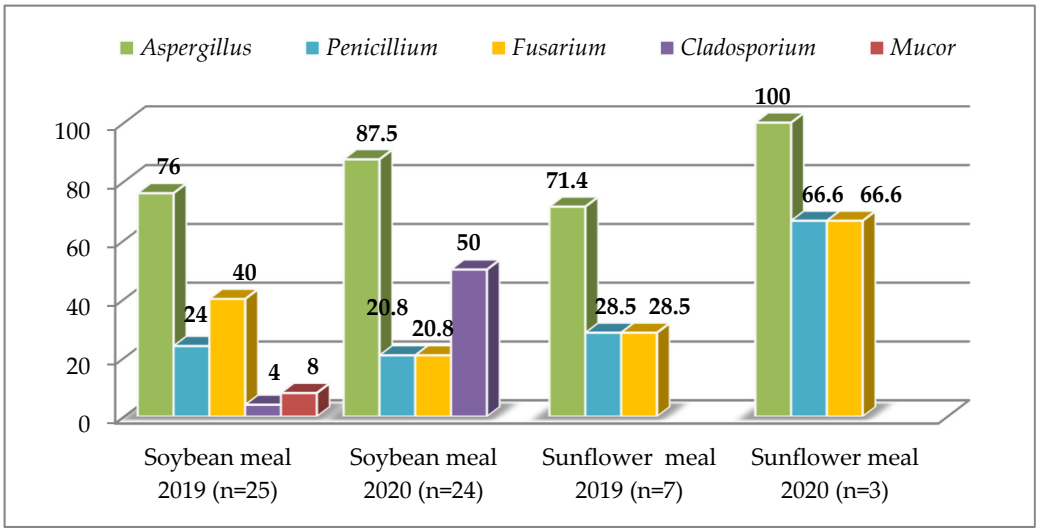

Figure 2. Proportion (%) of potentially toxigenic fungal genera in positive soybean and sunflower meal samples (n—number of positive samples).

3.2. Compound Feed

The results of microbiological assessment (yeasts and molds, *Salmonella* spp., and *E. coli*, and *Clostridium perfringens*) of broiler compound feed (starter, grower, and finisher) are presented in Table 2.

The mean values established for the analysis to determine the yeast and mold contamination of the starter compound feed were 5.9×10^2 cfu/g in 2019 for 50% of the positive samples and a maximum value of 2.9×10^3 cfu/g, and 5.3×10^2 cfu/g in 2020 for 42.8% of the positive samples with a maxim value of 9.0×10^2 cfu/g. In the case of grower compound feed, a proportion of 38.4% of positive samples was identified in 2019 and 44.8% in 2020, and also the average value of 4.2×10^2 cfu/g in 2019 respectively 6.5×10^2 cfu/g in 2020. Regarding finisher compound feed, positive samples had a percentage of 37.5% in 2019 with a mean value of 4.2×10^2 cfu/g, and 40.5% in 2020 with a mean of 5.8×10^2 cfu/g. The proportions of potentially toxigenic fungi genera identified in the starter (Figure 3), grower (Figure 4), and finisher (Figure 5) compound feed highlight that the *Aspergillus* and *Penicillium* genera were predominantly isolated.

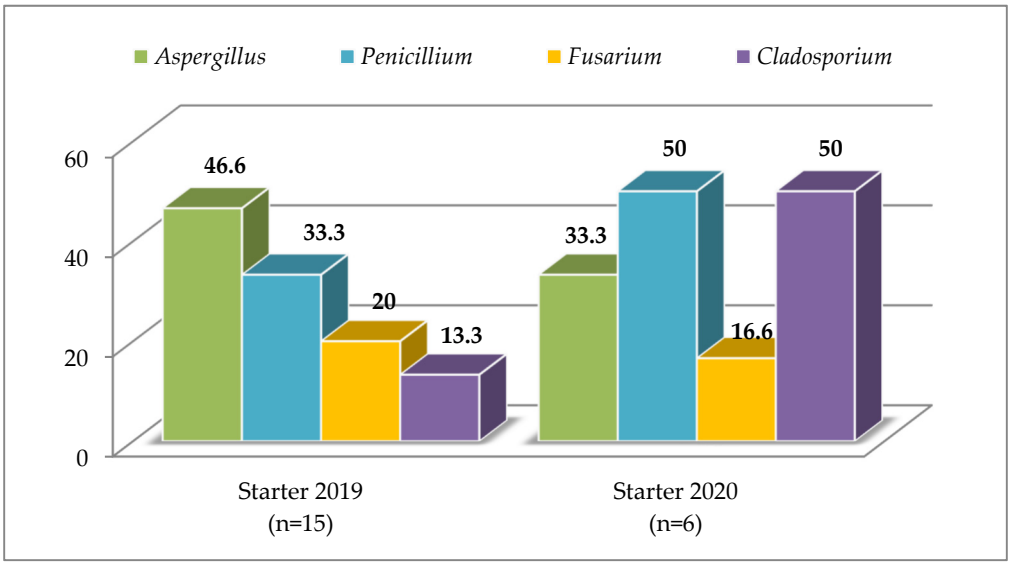

Figure 3. Proportion (%) of potentially toxigenic fungal genera in positive starter compound feed samples (n—number of positive samples).

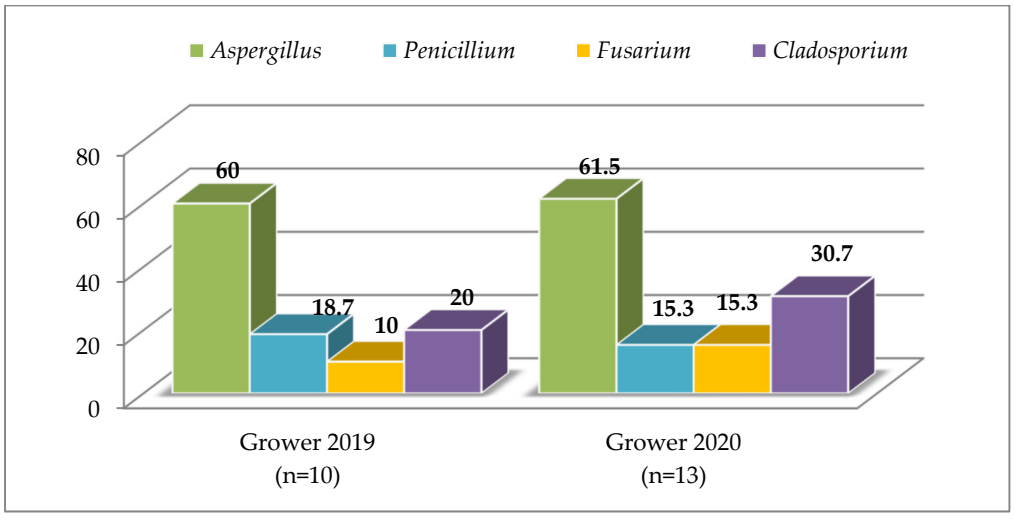

Figure 4. Proportion (%) of potentially toxigenic fungal genera in positive grower compound feed samples (n—number of positive samples).

Table 2. Results of microbiological assessment of compound feed.

Specification		2019 Year						2020 Year					
		n	Positive (%)	$\bar{x}$	s	Min.	Max.	n	Positive (%)	$\bar{x}$	s	Min.	Max.
Yeasts and molds (cfu/g)	Starter	30	50.0	5.9×10^2	6.4×10^2	4.0×10^2	2.9×10^3	14	42.8	5.3×10^2	1.9×10^2	4.0×10^2	9.0×10^2
	Grower	26	38.4	4.2×10^2	6.3×10^1	4.0×10^2	6.0×10^2	29	44.8	6.5×10^2	6.4×10^2	4.0×10^2	26.5×10^2
	Finisher	48	37.5	4.2×10^2	3.3×10^1	4.0×10^2	7.0×10^2	37	40.5	5.8×10^2	5.1×10^2	4.0×10^2	23.5×10^2
Salmonella spp. /25 g	Starter	31	0	0	0	absent/25 g		14	0	0	0	absent/25 g	
	Grower	26	0	0	0	absent/25 g		27	0	0	0	absent/25 g	
	Finisher	48	0	0	0	absent/25 g		37	0	0	0	absent/25 g	
E.coli (cfu/g)	Starter	29	0	0	0	0	0	14	0	0	0	0	0
	Grower	25	0	0	0	0	0	27	0	0	0	0	0
	Finisher	47	0	0	0	0	0	37	0	0	0	0	0
Clostridium perfringens (cfu/g)	Starter	13	0	0	0	0	0	2	0	0	0	0	0
	Grower	13	0	0	0	0	0	1	0	0	0	0	0
	Finisher	24	0	0	0	0	0	2	0	0	0	0	0

n—number of samples analyzed. $\bar{x}$—mean. s—standard deviation. Min.—minimum value identified. Max.—maximum value identified.

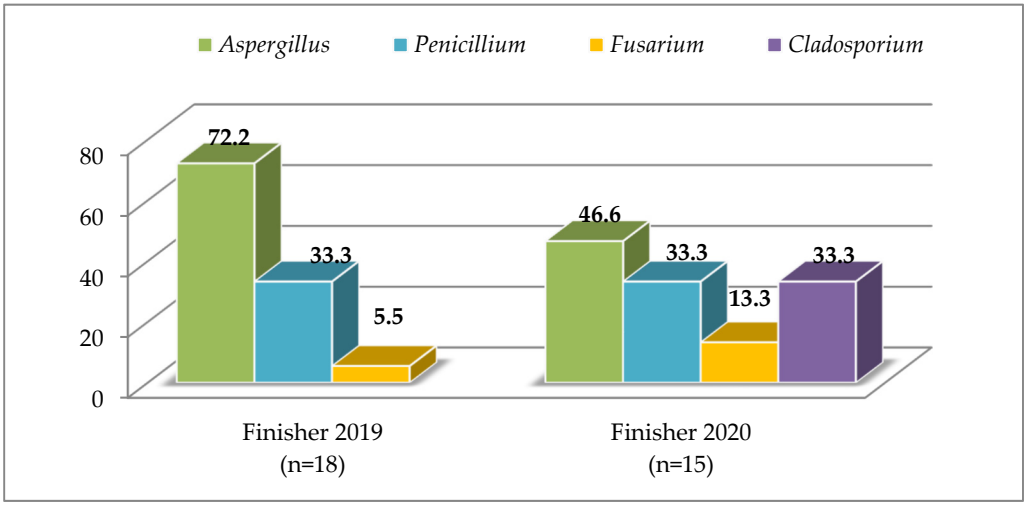

Figure 5. Proportion (%) of potentially toxigenic fungal genera in positive finisher compound feed samples (n—number of positive samples).

4. Discussion

4.1. Raw Materials

The main sources of fungal microflora in compound feed originate from raw materials of plant origin, firstly cereals [50]. Potentially toxigenic fungi are associated with oilseeds and cereals and mostly belong to the *Fusarium*, *Aspergillus*, and *Penicillium* genera [51]. It is distinguished in Figure 1 that the *Aspergillus* genus was identified in a high proportion of cases, over 50% in both years of research in contrast to the research carried out by Krnjaja et al. [52] which identified the most frequent genera as the *Fusarium* genera (92.22%). Krnjaja et al. [52] analyzed 127 maize samples for determinate fungal contamination; the total fungal count ranged from 1.0×10^1–3.0×10^6 cfu/g. In our study, the total fungal count ranged from 4.0×10^2 to 4.9×10^3 cfu/g. To determine the content of the yeasts and molds in the wheat samples, four analyses were carried out in both years of the study, with all of the results being positive. *Aspergillus* genera was isolated in more than 70% for the two types of meals analyzed in both years of the study (Figure 2). Studies have specified that fungi of the *Aspergillus* genera are the main producers of aflatoxins [53] and ochratoxin A, which in terms of their effect are the second most investigated mycotoxin after aflatoxins [54]. For the 60 most common mycotoxins occurring in feed, 48% have been shown to be produced by the *Fusarium* genus, 13% by the *Aspergillus* genus, 8% by the *Penicillium* genus, and 12% by the *Alternaria* genus [55]. In our study, high fungal colony counts for maize and wheat in both of the research years can be attributed to favorable climate conditions that were ideal for the growth of toxic mold during their growing and harvesting phases. The right temperature and humidity levels are essential for the growth of toxic fungus in grain both before and after harvest. The grain's moisture content is also one of the most crucial preconditions for grain infection during storage. A moisture level of 15% or less in maize is acceptable for safe storage.

Raw materials used in compound feed production represent an important source of *Salmonella* contamination [39]. Jones and Richardson [56] isolated *Salmonella* from maize, cottonseed meal, soybean meal, and wheat bran. In a raw materials survey, Ge B. et al. [57] identified positive samples for *Salmonella* contamination for 14 (54.2%) of 31 samples of soybean meal, 3 (60%) of 5 samples of sunflower meal, and 3 (17.6%) of 17 samples of maize. In our study, there were no positive results for *Salmonella* spp. contamination during the two years for the raw materials studied.

In addition, the results to determine raw materials contaminated with *E. coli* and *Clostridium perfringens* were negative. In their study, Da Costa et al. [58] analyzed 66 samples of raw materials (corn, wheat, barley, soybean meal, and sunflower meal) for *E. coli* contamination and identified that 89.2% of the samples were positive. Casagrande et al. [59]

demonstrated that out of the 80 raw materials' samples used in compound feed production, *C. perfringens* was isolated in 12.5% of the plant origin samples (soybean meal, and maize). Prió et al. [60] studied raw material microbial contamination and identified the presence of *C. perfringens* in 1.2% of maize samples (298 samples analyzed), 35.2% of wheat samples (85 samples analyzed), 5.3% of soybean meal samples (464 samples analyzed), and 13.3% of sunflower meal samples (70 samples analyzed). Another study conducted an analysis of 298 poultry feed ingredients and *C. perfringens* was detected in 33.89% of samples; the highest level of contamination with *C. perfringens* was observed in fish meal (55.26%) followed by bone meal (44.83%); the same as in our study, negative results of *C. perfringens* contamination were noticed in soybean meal and maize [61].

Regarding the correlation in the microbiological analysis results for raw materials intended for compound feed, all of them were in accordance with the limits allowed by legislation [62,63].

4.2. Compound Feed

Dalcero et al. [64] found the highest incidence of the *Aspergillus* (85%) and *Fusarium* genera (70%) in 130 samples of poultry feed. Rosa et al. [65] collected 96 samples of poultry feeds from four feed mills which were examined for total molds and for *Aspergillus* and *Penicillium* occurrence; the total mold counts were generally higher than 1.0×10^5 cfu/g; the *Aspergillus* and *Penicillium* species were isolated in the highest numbers. Shareef [66] realized a study in which 45 samples of poultry feed were analyzed; the most frequent fungi were from genus *Aspergillus* (88%) with a range of 1.0×10^4–5.3×10^6 cfu/g and a mean value of 2.6×10^6 cfu/g and the second most frequent were the from the genera *Penicillium* and *Mucor* (64%) with a range of 2.0×10^4–4.4×10^6 cfu/g and 3.0×10^4–2.6×10^5 cfu/g and a mean of 2.2×10^6 and 1.4×10^5 cfu/g, respectively. In a study realized by Cegielska-Radziejwska et al. [67], 45 samples of compound feed for broilers were analyzed, collected from four different feed mills in Poland; the samples consisted of compound feed for the different growing stage of broilers (starter, grower, and finisher). For the starter compound feed, the yeasts and molds ranged from 5.5×10^1–3.0×10^2 cfu/g and a mean value of 1.8×10^2 cfu/g; the determined grower compound feed values ranged from 6.0×10^1–4.7×10^2 cfu/g with a mean of 3.2×10^2 cfu/g; the finisher compound feed had values for yeasts and molds ranging from 8.5×10^1–7.0×10^3 cfu/g and a mean of 1.6×10^3 cfu/g. In the tested feed samples, fungi from the genera *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, and *Rhizopus* were identified, with *Aspergillus* being the most commonly found genera, the same as in our study (Figures 3–5). A total of 49 samples of compound feed for broilers were analyzed by Greco at al. [68] for mycotoxigenic fungi and the most frequent mycotoxigenic fungi were those from the genus *Fusarium* (69.6), followed by *Eurotium* (52.2%), *Penicillium* (45.6%), and *Aspergillus* (43.5%). The fungal count for the compound feed was typically lower than the values found for raw materials. The pelleting procedure was applied to the studied compound feed, and it has been established that this procedure considerably reduced the fungal counts. As a result, pelleting may be the cause of the moderate frequency of some fungal genera.

Contamination of compound feed with *Salmonella* has been considered as an important source of contamination for broilers since the 1950s [69]. The potential transmission of *Salmonella* strains from feed to animals and subsequently to carcasses has been proven since the 1970s [70]. Over a period of 16 years, 23,963 samples for *Salmonella* culture and serotyping were collected from 22 stock feed mills in Australia. The percentage of positive samples ranged from 7.2% in 2003 to 2.8% in 2017. Of the 1069 positive samples, 976 were serotyped with 61 different *Salmonella* serotypes were isolated. The serotype most frequently isolated from raw materials was *Salmonella* Agona, (n = 108) whilst *Salmonella* Anatum was the serotype most frequently isolated from compound feed (n = 156) [71]. To determine *Salmonella* contamination in the studied broiler compound feeds, 105 analyses were carried out in 2019, and 78 analyses were carried out in 2020, with all of the results being negative, in accordance with the legislative regulations [62,63].

To determine the compound feed contamination with *E. coli*, 101 analyses were carried out in 2019, and 78 analyses were carried out in 2020, with all of the results being negative (Table 1). A survey on the prevalence of *Escherichia coli* in compound feed was carried out over a period of nine years in the Republic of Croatia; a total of 1688 feed samples were collected from feed mill and poultry farms, and analyses showed the presence of *E. coli* in 629 (37.3%) feed samples, which is different to our results [72]. In their study, Da Costa et al. [58] analyzed 23 samples of broiler compound feed for *E. coli* contamination and it was detected that 100% of the samples were positive. A study conducted an analysis on 378 animal feed samples to determine the prevalence of *E. coli* isolated from U.S. feed between 2005–2011; from the 65 samples of the poultry feed analyzed, 22 were positive (33.8%) [73].

In a survey conducted by Schocken-Iturrino et al. [74], they observed that among the 90 samples of broiler compound feed analyzed, 42% were contaminated by *C. perfringens*, and the mean count was 3.69×10^2 cfu/g. Tessari et al. [75] analyzed 121 samples of compound feed and reported *C. perfringens* isolated in 23 samples (19%). In comparison with the previous study, in our study all of the results were negative for *C. perfringens* contamination.

For the negative results of microbiological analysis (*Salmonella* spp., *E. coli*, and *C. perfringens*) both for the raw materials and compound feed, maybe it is because the feed mill that was studied has implemented a HACCP (hazard analysis and critical control points) system, which, as specified in Regulation (EC) number 183 of 2005 [43], can facilitate the achievement of a high level of feed safety; this may be a reason as to why our results are different from other studies.

Regarding the correlation in the microbiological analysis results for compound feed, the same as in case of raw materials, all of them were in accordance with limits allowed by national legislation [63,64].

5. Conclusions

Microbiological control of raw materials and broiler compound feed must be considered relevant due to the demands of consumers for food safety all over the food chain. This fact is possible through the introduction of an appropriate system for monitoring and analyzing microbiological contaminants in feed mill; this contributes to the control and prevention of contamination, with it having a direct impact on food safety and animal and human health. Based on the results obtained of the study, it can be concluded that the microbiological analysis is justified and necessary to assess the safety of broiler compound feed. It is necessary to continue the assessment with a complete approach to the hazards associated with food safety (physical, chemical, and biological), extended to other raw materials and categories of compound feed.

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