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STUDY ON ANALYSIS OF BIOLOGICAL HAZARDS ASSOCIATED WITH COMPOUND FEED PRODUCING IN RELATION ON FOOD SAFETY

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Abstract

Compound feed and raw materials are potential vectors of pathogenic bacteria and potentially toxigenic fungi. The paper conducts a study on the production of compound feed in relation on food safety, by mycological and bacteriological analysis of samples of raw materials and compound feed for broilers, taken from a feed mill from Romania during 2019. Methodologically, the data were processed, analyzed and synthesized in the form of graphs and tables. The obtained results highlight during the analyzed period, in the samples of mycologically analyzed raw materials, eight genera of potentially toxigenic fungi were identified; the largest presence was the genus Aspergillus (64.45%). In the analyzed compound feed samples, four potentially toxigenic fungal genera were identified; in the samples with quantifiable results, the majority (61.9%) was identified the genus Aspergillus, and the lowest presence was the genus Cladosporium (9.53%). All results of bacteriological analysis aimed at determining the contamination with Salmonella spp. (54 analyzes for raw materials and 105 for compound feed) and E. coli (51 analyzes for raw materials and 101 for compound feeds) were negative. It can be concluded that in the production process of compound feed, the mycological, bacteriological and mycotoxicological analysis is must both for raw materials susceptible to contamination and of the finished products obtained; this goal is achieved in the unit studied, the results highlight the effectiveness of specific food safety control processes.

Key words*: feed safety, food safety, toxigenic fungi, Salmonella spp.*

INTRODUCTION

Feed safety is an important prerequisite for obtaining optimal production results as well as for maintaining the health of animals, especially in intensive industrial production, so it is necessary to constantly monitor raw materials and compound feed (Krnjaja et al., 2010). Compund feed and raw materials can be contaminated with undesirable substances, which may come from the environment or the production process (EFISC, 2014). Due to the relevant role of compound feed industry in the food chain, to ensure their safety, EC Regulation no. 183/2005 specifies, through Article 6 (1), that "Feed manufacturers shall establish, apply and maintain one or more permanent written procedures based on the HACCP principles."

Raw materials used to produce compound feed can come from various locations (Davies & Wales, 2013); if there has been exposure to wild or fecal animals, they may act as a source of non-endemic serotypes of *Salmonella* and other enteric bacteria, including the pathogenic *Escherichia coli* (Gosling et al., 2021). *Salmonella* can persist for many years in dry environments, such as those in feed mills, grain depots, and feed bunkers, and once it becomes resident, it can be difficult to eradicate (Davies and Wray, 1997). The presence of pathogens in compound feed may occur due to the use of contaminated raw materials during transport, in the production unit or on site. Because bacterial contaminants are unevenly distributed in the feed, the bacteria present may be damaged and difficulties may occur during microbial analysis. The purpose of controlling feed pathogens should be to ensure that feed contaminants are below a critical threshold to minimize the risk to human and animal health (Alali & Ricke, 2012).

Microbial contamination of feed is a potentially significant route for the entry of pathogens, including *Campylobacter* species, *Salmonella* enterica serotypes, *Escherichia coli* strains and *Yersinia enterocolitica*, into the human diet. Food-producing animals can be infected and

colonized with pathogens by ingesting contaminated feed; they can then be transmitted through the food chain to humans (Huss et al., 2015).

Contamination with potentially toxigenic fungi of feed is a regular occurrence worldwide and harmful effects have been observed in all classes of farm animals due to the production of mycotoxins by certain species and mold strains (D'Mello, 2004). Potentially toxigenic fungi are associated with cereals and oilseeds and mainly belong to the genera *Fusarium*, *Aspergillus* and *Penicillium* (Pacin, 2002). Factors that influence the development of microorganisms are represented by temperature, oxygen, relative humidity, water activity, pH, nutrients and different types of inhibitors (Savu & Georgescu, 2004).

Animals can become infected when they are fed compound feed contaminated with *Salmonella*; this can cause occasional clinical disease in some animals, but the major result is asymptomatic transmission. In addition, animals be infected by other animals infected with *Salmonella*, directly or through a contaminated environment for which the original source may have contaminated feed. *Salmonella* has been shown to be transmitted from feed to animals that consume it, and subsequently in food (EFSA, 2008). *E. coli* is a ubiquitous bacterium, present naturally in the human digestive tract in vast numbers, only a few strains being pathogenic and can induce symptoms; this serotype is of great importance for human and veterinary pathology, human becoming one of the most dangerous etiological agents of food poisoning (Savu & Georgescu, 2004).

Regulation (EC) no. Regulation (EC) No 2160 of 2003 on the control of *Salmonella* and other specific zoonotic agents present in the foodstuffs ensures that appropriate and effective measures are taken to detect and control them at all relevant stages of production, processing and distribution, including feed, to reduce their prevalence and the risk they pose to public health. In accordance with Article 5 (3) of Regulation (EC) No 183 of 2005 on feed hygiene, feed manufacturers must comply with specific microbiological criteria.

The paper conducts a study during 2019, on the production of compound feeds in relation to food safety, by mycological and bacteriological analysis of raw materials and compound feeds for broilers.

MATERIALS AND METHODS

Methodologically, the results of mycological and bacteriological analyzes performed throughout 2019 on raw materials and finished products from a compound feed factory in Romania were processed, synthesized and interpreted. Mycological analysis determined the content of raw materials and compound feeds in yeasts and molds, and bacteriological analysis determined the degree of contamination of raw materials and compound feed with *Salmonella* spp. and *E. coli*.

To obtain relevant results regarding the production of compound feed in relation on food safety, some raw materials susceptible to contamination (maize grain, wheat grain, soybean meal, sunflower meal) and the finished products obtained were analyzed, respectively, compound feed for broiler in different growth phases (starter, grower, finisher).

The analysis was performed in accredited specialized laboratories according to the following methods: SR ISO 21527–2: 2009 Microbiology of food and feed - Horizontal method for enumeration of yeasts and molds - Part 2: Technique for counting colonies in products with higher water activity less than or equal to 0.95; SR EN ISO 6579–1: 2017 Microbiology of the food chain. Horizontal method for the detection, counting and serotyping of S*almonella*. Part 1: Detection of *Salmonella* spp.; SR ISO 7251: 2009 Food microbiology; Horizontal method for the detection and enumeration of presumptive *Escherichia coli;* The least likely number technique.

The results obtained were compared with the values regulated by national and European legislation. The interpretation of the results led to the formulation of conclusions concerning the production of compound feeds in relation to food safety.

RESULTS AND DISCUSSIONS

The results of the mycological and bacteriological analysis performed for the samples of raw materials (corn grain, wheat

grain, soybean meal, sunflower meal) taken from the feed mill studied (Table 1), were presented for each month from January - December 2019.

Table 1. Results of biological analysis for raw materials

Specification		n[1]	MYCOLOGICAL ANALYSIS RESULTS		BACTERIOLOGICAL ANALYSIS RESULTS	
			Yeasts and molds (cfu/g) max. 5 x 10^3		*Salmonella* spp. max. absent/25 g	*Escherichia coli* cfu/g max. 10^2 cfu/g
			m[2] - M[3]	$\bar{x}$[4]		
Maize grain	Jan.	2	400	-	absent/25 g	-
	Feb.	3	4300	-	absent/25 g	0
	Mar.	6	700 - 1300	1000	absent/25 g	0
	Apr.	-	-	-	-	-
	May	5	900 - 3400	1966	absent/25 g	0
	June	-	-	-	-	-
	July	8	400 - 600	550	absent/25 g	0
	Aug.	-	-	-	-	-
	Sep.	4	800 - 1000	900	absent/25 g	0
	Oct.	1	800	-	-	-
	Nov.	2	800 - 3300	2050	-	-
	Dec.	1	400	-	-	-
Wheat grain	Jan.	2	400	-	absent/25 g	-
	Feb.	3	400	-	absent/25 g	0
	Mar.	6	600 - 2400	1500	absent/25 g	0
Soybean meal	Jan.	11	400 - 1000	625	absent/25 g	0
	Feb.	15	400 - 2900	980	absent/25 g	0
	Mar.	6	500 - 1000	750	absent/25 g	0
	Apr.	3	600	-	absent/25 g	0
	May	11	100 - 3400	1800	absent/25 g	0
	June	3	400	-	absent/25 g	0
	July	8	400 - 1636	1018	absent/25 g	0
	Aug.	13	400	400	absent/25 g	0
	Sep.	6	100 - 900	500	absent/25 g	0
	Oct.	3	500	-	absent/25 g	0
	Nov.	9	400 - 1550	850	absent/25 g	0
	Dec.	2	-	-	absent/25 g	0
Sunflower meal	Jan.	3	700	-	absent/25 g	0
	Feb.	6	400 - 800	600	absent/25 g	0
	Mar.	3	1600	-	absent/25 g	0
	Apr.	-	-	-	-	-
	May	2	-	-	absent/25 g	0
	June	3	1900	-	absent/25 g	0
	July	2	-	-	absent/25 g	0
	Aug.	-	-	-	-	-
	Sep.	3	400	-	absent/25 g	0
	Oct.	-	-	-	-	-
	Nov.	-	-	-	-	-
	Dec.	2	-	-	absent/25 g	0

[1]n = number of analysis
[2]m = minimum
[3]M = maximum
[4]$\bar{x}$ = average

The values for the quantitative determination of the contamination with yeasts and molds of the sampled corn grains varied between 400 cfu/g and 4300 cfu/g (n = 17), with an average of 1293 cfu/g, being below the maximum limit allowed by legislation (max. 5 x 10^3 cfu/g - the total amount of potential toxin-producing fungal species, according to Order MAFF 249/2003); for two analyzed samples (11.76%) the results were undetectable. All the results of the bacteriological analyzes (*Salmonella* spp. - n = 8 and *E. coli* - n = 7) performed for the maize grains registered negative values, being in accordance with the limits imposed by the legislation (absent/25 g *Salmonella* spp. and max. 10^2 cfu/g Escherichia coli according to Order MAFF 249/2003).

The results of mycological analyzes (n = 4) performed for wheat grains had values below the maximum limit imposed by the legislation, between 400 cfu/g and 2400 cfu/g, with an average of 950 cfu/g. All the results of the

bacteriological analyzes (*Salmonella* spp. - n = 4 and *E. coli* – n = 3) performed for the wheat grains registered negative values, being in accordance with the limits imposed by the legislation (absent/25 g *Salmonella* spp. and max. 10^2 ufc/g *E. coli*).

The resulting values for the concentration of yeasts and molds in soybean meal samples were below the limit imposed by the legislation in force (5 x 10^3 cfu/g), ranging between 400 cfu/g and 3400 cfu/g (n = 27), with an average of 865 ufc/g; for one analyzed sample (3.7%) the result was unquantifiable. All results of bacteriological analyzes to determine contamination with *Salmonella* spp. (n = 32) and *E. coli* (n = 31) were negative, in accordance with the maximum limits imposed by legislation (absent/25 g *Salmonella* spp. and max. 10^2 cfu/g *E. coli*).

The results for determining the content of yeasts and molds in sunflower meal had values below the maximum limit imposed by legislation, between 400 cfu/g and 1900 cfu/g (n = 7) with an average of 1040 cfu/g. All results of bacteriological analyzes to determine contamination with *Salmonella* spp. (n = 10) and *E. coli* (n = 10) were negative, in accordance with the maximum limits imposed by legislation.

According to the graphical representation of the number of potentially toxigenic fungal genera identified for each raw material analyzed (Figure 1), the content in the genus *Aspergillus* was predominant (65.45%) in all raw materials, followed by the content in the genus *Fusarium* (41.81%). The literature specifies that fungi of the genus *Aspergillus* are the main producers of aflatoxins (Marin et al., 2013), and ochratoxin A, the second most investigated mycotoxin after aflatoxins, in terms of its effect (Völkel et al., 2011).

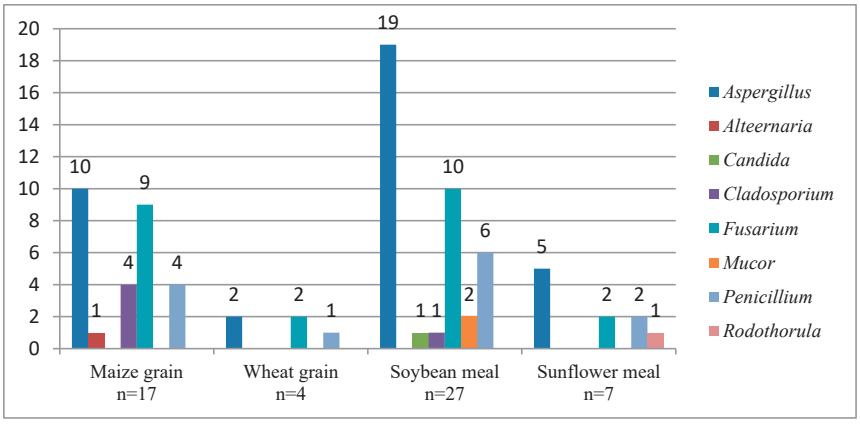

Figure 1. Number of fungal genera identified in raw materials

The results of mycological and bacteriological analyzes performed for the samples of compound feeds (starter, grower, finisher) from feed mill studied (Table 2), were presented for each month in which analyzes were performed, from January to December 2019.

The values found for the quantitative determination of the contamination of starter compound feed with yeasts and molds, varied between 100 cfu/g and 2900 cfu/g (n = 30), resulting in an average of 507.6 cfu/g, in accordance with the maximum regulated limits (max 5 x 10^3 cfu/g - the total number of potentially toxin-producing fungal species according to Order MAFF 249/2003); for 16 samples analyzed (53.3% of the total) the results were undetectable.

Table 2. Results of biological analysis of compound feed

Specification		n[1]	MYCOLOGICAL ANALYSIS RESULTS		BACTERIOLOGICAL ANALYSIS RESULTS	
			Yeasts and molds (cfu/g) max. 5 x 10^3		*Salmonella* spp. max. absent/25 g	*Escherichia coli* cfu/g max. 10^2 cfu/g
			m[2] - M[3]	$\bar{x}$[4]		
Starter compound feed	Jan.	8	100 - 2900	1133	absent/25 g	0
	Feb.	6	400	400	absent/25 g	0
	Mar.	3	nd[5]	-	absent/25 g	0
	Apr.	12	100 - 400	325	absent/25 g	0
	May	15	100 - 400	280	absent/25 g	0
	June	3	800	-	absent/25 g	0
	July	6	nd	-	absent/25 g	0
	Aug.	10	400	-	absent/25 g	0
	Sep.	9	nd	-	absent/25 g	0
	Oct.	6	400	400	absent/25 g	0
	Nov.	9	nd	-	absent/25 g	0
	Dec.	3	400	-	absent/25 g	0
Grower compound feed	Jan.	5	nd	-	absent/25 g	-
	Feb.	9	400	400	absent/25 g	0
	Mar.	9	400 - 600	500	absent/25 g	0
	Apr.	3	400	-	absent/25 g	0
	May	9	400	-	absent/25 g	0
	June	3	nd	-	absent/25 g	0
	July	9	400	400	absent/25 g	0
	Aug.	3	nd	-	absent/25 g	0
	Sep.	6	nd	-	absent/25 g	0
	Oct.	-	-	-	-	-
	Nov.	12	400	400	absent/25 g	0
	Dec.	9	nd	-	absent/25 g	0
Finisher compound feed	Jan.	8	400	-	absent/25 g	0
	Feb.	21	400	400	absent/25 g	0
	Mar.	24	400	-	absent/25 g	0
	Apr.	6	400	-	absent/25 g	0
	May	12	400	-	absent/25 g	0
	June	9	nd	-	absent/25 g	0
	July	18	400	-	absent/25 g	0
	Aug.	9	nd	-	absent/25 g	0
	Sep.	24	400 - 700	475	absent/25 g	0
	Oct.	-	-	-	-	-
	Nov.	6	400 - 500	450	absent/25 g	0
	Dec.	6	500	-	absent/25 g	0

[1]n = number of analysis; [2]m = minimum; [3]M = maximum; [4]$\bar{x}$ = average; [5]nd = not detectable

All results of bacteriological analyzes to determine *Salmonella* spp. (n = 31) and *E. coli* (n = 29) for starter compound feeds had negative values, in accordance with the limits allowed by law (absent/25 g *Salmonella* spp. and max. 10^2 cfu/g *E. coli* according to Order MAFF 249/2003).

The results for determining yeasts and molds in grower compound feed, had values between 400 cfu/g and 600 cfu/g (n = 26) with an average of 416 cfu/g. All results of bacteriological analyzes to determine contamination with *Salmonella* spp. (n = 26) and *E. coli* (n = 25) were negative. The values for the quantitative determination of finisher compound feed with yeasts and molds, varied between 100 cfu/g and 700 cfu/g (n = 40), with an average of 380 cfu/g; for 30 samples analyzed (62.5% of the total) the results were undetectable. All results of bacteriological analyzes to determine the contamination with *Salmonella* spp. (n = 48) and *E. coli* (n = 47) performed for compound feeds recorded negative values.

The graphical representation of the number of potentially toxigenic fungal genera identified (Figure 2) for each type of compound feed (starter, grower, finisher) analyzed, reveals that the genus *Aspergillus* was most often identified (61.9%) in the samples that had results quantifiable, followed by the genus *Penicillium* (33.3%).

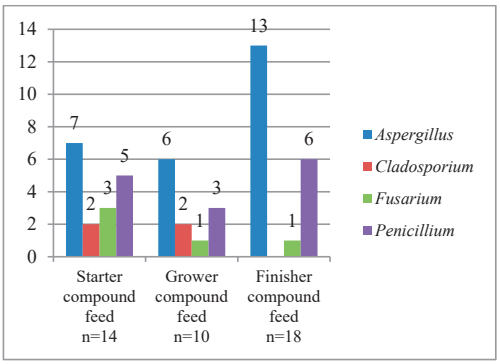

Figure 2. Number of fungal genera identified in compound feed

CONCLUSIONS

Compound feed and raw materials represent a favorable environment for the development of potentially toxigenic fungi and bacteriological contaminants, and therefore permanent analysis is needed to control them.

In the samples of raw materials analyzed, eight genera of potentially toxigenic fungi were identified: *Aspergillus*, *Alternaria*, *Candida, Cladosporium*, *Fusarium*, *Mucor*, *Penicillium* and *Rodothorula*; the highest presence was the genus *Aspergillus* (64.45%) followed by the genus *Fusarium* (41.81%), and the lowest presence was the genus *Rodothorula* (1.81%).

In the samples of compound feed analyzed, four genera of potentially toxigenic fungi were identified: *Aspergillus*, *Cladosporium*, *Fusarium* and *Penicillium*; the most present was the genus *Aspergillus* (61.9%), and the lowest presence was the genus *Cladosporium* (9.53%).

Regarding the correlation of the results of mycological analyzes with the maximum limits allowed by the legislation, it was found that they were within the regulated values, both for raw materials and for compound feeds.

Regarding the results of bacteriological analyzes aimed at determining the contamination with *Salmonella* spp. and *E. coli*, for raw materials and compound feed studied no positive values were registered.

Although no results were found above limits allowed by the legislation, the frequency of identification of mycotoxin-producing fungal genera is not negligible.

It can be concluded that in the production process of compound feeds, the mycological, bacteriological and mycotoxicological analysis is must both for raw materials susceptible to contamination and of the finished products obtained, in order to ensure public health; this goal is achieved in the unit studied, the results highlighting the effectiveness of specific food safety control processes.

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