# *SUMMARY*

**Key words: compound feed, raw materials, food safety, HACCP, food chain**

The solution for maintaining collective public health is the systematic control of hazards throughout the food chain, from “farm to fork”.

The global food system is facing profound changes in consumption patterns, in particular by increasing demand for animal products, which has led to increased global intensive animal production. But then, there is a growing need for raw materials used in the production of feed, corresponding to the nutritional requirements of animals and healthy, without unwanted substances that could compromise the quality and safety of food of animal origin.

The doctoral thesis entitled **“*Research on the production of compound feed in relation on food safety*”** is structured in two parts: the bibliographic study part and the personal research part, summing up nine chapters to which were added the conclusions and recommendations, originality and innovative contributions of thesis and bibliography.

Part I of the thesis included four chapters that present notions about the production of compound feed, elements of food quality and safety management, the bibliographic study on the assessment of physical, chemical and biological hazards associated with the production of compound feeds in relation on food safety and literature research.

Part II of the thesis, structured in five chapters, contains the purpose and organization of research, materials and methods, the results obtained on the application of HACCP system in the studied units, and results obtained on the analysis and control of physical, chemical and biological hazards associated with the production of compound feeds in relation on food safety.

The aim of the doctoral thesis was to highlight how the HACCP system can contribute to the safe production of compound feed for broiler in different growth stages, by applying its principles and stages on the feed production flow.

To achieve the proposed aim, a case study was undertaken to highlighting the role of the HACCP system as a food safety factor, and the extent to which it can respond favorably to the interconnection between safe production of compound feed and obtaining of safe food of animal origin for the end consumer.

In order to achieve the proposed goal, two feed mills representative for Romania in terms of production capacity (70,000 - 120,000 t/year each), named in the paper "unit A" and "unit B" were studied.

The results obtained in the research carried out during 2019 and 2020 in two feed mills (unit A, respectively unit B), allowed the formulation of some conclusions, summarized below:

– the application of the **HACCP** system in the two feed mills studied during the twelve specific stages was analyzed; thus it was possible to observe the high degree of specificity of the system.

– the **physical hazards** associated with the production of compound feed from the two units studied during 2019 and 2020 were analyzed and controlled. Regarding the physical hazards identified in **unit A**, it was found that in 2019 at the stage of receipt of raw materials, 20 batches of maize grains (68.9%) and 9 batches of wheat grains (31%) were refused, the most frequently identified danger (78.5%) being represented by the high humidity of the grains. In 2020, 17 batches of raw materials were refused, represented by maize grains (52.9%) and wheat grains (47%); also, humidity was the predominantly identified potential hazard (70.5%). Comparatively, in 2019 there were more refusal of raw materials (n=29) based on physical parameters, compared to 2020 (n=17).

Regarding **unit B**, based on the physical parameters identified in the stage of reception of raw materials in 2019, 22 batches of maize grains (91.6%) and two batches of wheat grains (8.3%) were refused. ); the most frequently identified danger (58.3%) was the presence of sprouted and moldy grains. In 2020, 53 batches of raw materials were rejected, represented by corn grains (79.2%) and wheat grains (20.7%); the most frequently identified danger was live beetle infestation (50.9%). In comparison, in 2020, 54.7% more non-compliant products were registered than in 2019.

– a series of **chemical hazards** associated with the production of compound feed in relation to food safety, represented by mycotoxins, heavy metals and polychlorinated biphenyls, were evaluated and controlled; the incidence of hazards in raw materials (maize grain, wheat grain, soybean meal, sunflower meal) and compound feeds intended for feeding broiler in different growth phases (starter, grower, finisher) in both units studied was determined, on during the years 2019 and 2020. In mycotoxicological analyzes, the content of raw materials and finished products in the following mycotoxins was determined: total aflatoxin (AFT), aflatoxin $B_1$ (AFB1), deoxynivalenol (DON), fumonizin $B_1$+$B_2$ (FUM), ochratoxin A (OTA), T-2 toxin (T-2) and zearalenone (ZEN), the results being discussed separately for each unit:.

**Unit A**

For the analyzes performed on grain maize at the reception of raw materials in 2019, the percentage of positive samples ranged between 60%

(OTA) and 92.8% (DON); in fact, the incidence was over 60% for all six types of mycotoxins analyzed. Compared to the maximum value allowed by law, the value closest to it was identified in the case of AFT (14 ppb). In 2020, the proportion of positive samples was over 70% for all types of mycotoxins analyzed; also, the maximum value closest to the limit allowed by legislation was also identified for AFT (7.3 ppb).

Regarding the analyzes performed at the reception of raw materials for the quantitative determination of mycotoxins in wheat grains, in 2019 an incidence between 60% (FUM) and 100% (T-2) was identified; the maximum value closest to the regulated limit was found for the concentration in AFT (8 ppb). In 2020, an incidence was identified between 68.4% (AFT) and 100% (FUM, OTA, ZEN) and, like the previous year, the value closest to the legislative limit was identified on the content in AFT (6.2 ppb).

Soybean meal taken from the reception stage and analyzed mycotoxicologically had an incidence between 40% (FUM) and 100% (OTA, T-2, ZEN) in 2019, respectively between 88.8% (OTA) and 100% ( FUM, T-2) in 2020; the concentrations closest to the legislative limit were identified for AFT for both years of study (1.7 ppb and 2.8 ppb, respectively).

From the point of view of the maximum permitted levels and the indicative levels established by legislation, following the analyzes performed for the raw materials in Unit A, it was found that they were not exceeded.

The results obtained in unit A regarding the analysis of the mycotoxin concentration of the starter compound feed, revealed an incidence between 85.7% (FUM) and 100% (OTA, ZEN) in 2019, respectively between 76.4% (OTA ) and 100% (T-2) in 2020. The maximum values identified and the average values established for the mycotoxin content of starter feeds were below the limits established by law.

For the grower compound feed analyzed mycotoxicologically in 2019, the percentage of positive samples for the six types of mycotoxins analyzed was between 77.7% (FUM) and 100% (OTA, T-2, ZEN); in 2020, an incidence was set between 87.5% (ZEN) and 100% (DON, FUM, OTA).

In the case of finisher compound feed analyzed in 2019, an incidence of contamination was established which varied between 85% (T-2) and 100% (DON, OTA, ZEN); for 2020 the proportion of positive samples was between 78.9% (T-2) and 100% (ZEN).

It should be mentioned that all the maximum values together with the average values established in two years of study for the mycotoxicological analysis of the compound feed from unit A, were below the maximum and indicative limits provided in the legislation.

**Unit B**

For the determination of the total aflatoxin content of grain maize taken from the receiving stage of unit B, incidence studies indicated that in 2019, of the 1034 samples analyzed, 77.3% (n = 800) were positive, while in 2020, of the 1191 samples analyzed, 57.9% were positive; comparatively, a lower prevalence was identified in 2020, compared to 2019. Regarding the non-compliance of the results with the maximum limits allowed by legislation, in 2019 an excess was identified (70.4 ppb), and in 2020 they were found nine exceedances, with values between 30.1 ppb and 62.1 ppb.

For samples of wheat grains taken from the receiving stage and from the stock of unit B, analyzes were performed to determine the concentration in AFT and DON. For the grains taken from the reception stage, the established incidence was 66.9% (AFT) and 90.4% (DON) for 2019, respectively 51.2% (AFT) and 85.9% (DON).

Mycotoxicological analyzes for soybean meal and sunflower meal involved the determination of concentration in AFT, DON, FUM, OTA and ZEN. For the soybean meal analyzed, in 2019 there was an incidence of positive samples between 69.2% (FUM) and 100% (ZEN), and in 2020 the incidence was between 70% (FUM) and 100% (ZEN ); there is an increased incidence in both years of study for the concentration in ZEN. For sunflower meal, the proportion of positive samples ranged from 60% (FUM) to 100% (OTA) in 2019, respectively 78.5% (FUM) and 100% (DON, ZEN) in 2020; there is an increased incidence of OTA contamination in both years of the study.

The results obtained in 2019 regarding the determination of the mycotoxin content of the starter compound feed in unit B, revealed an incidence of: 70.6% (AFT), 89.1% (DON), 77.1% (FUM), 82.6% (OTA) and 94.5 (ZEN); on the other hand, the index identified in 2020 was: 57.1% (AFT), 98.9% (DON), 51.5% (FUM), 61.5% (OTA) and 90.1% ( ZEN).

For grower compound feed analyzed mycotoxicologically in 2019, an incidence of: 68.3% (AFT), 87.3% (DON), 69.6% (FUM), 79.7% (OTA) and 93 was identified, 6% (ZEN); comparatively, in 2020 the incidence of contamination was: 49.4% (AFT), 96.5% (DON), 43.6% (FUM), 52.8% (OTA), 90.8% (ZEN ).

Regarding the finisher compound feed, the proportion of positive samples was between 64.9% (AFT) and 91.9% (ZEN) for the analyzes performed in 2019, respectively between 51.04% (AFT) and 98.6% (DON) for the analyzes performed in 2020.

With regard to the mycotoxin concentration of feed in Unit B, it was found that all identified maximum values and established mean values were

lower than the maximum permitted levels and indicative levels established by regulations.

In order to determine the content in heavy metals (As, Cd, Cu, Pb, Zn,) in both years of study were analyzed starter and finisher compound feed from unit B. In 2019, for the analyzed starter compound feed, were found quantifiable results for Cd content (0.146 ppm), Pb (1.6 ppm) and Zn (14.1 ppb); for the finisher compound feed, positive values were identified for the content in Cu (10.6 ppm), respectively Zn (10.6 ppm). In 2020, quantifiable results for the concentration in As (0.2 ppm), Cu (13.4 ppm) and Zn (43.4 ppm) were identified for the finisher compound feed. All the results recorded were below the maximum limits imposed by law.

Regarding the results of the analyzes for the determination of the concentration in polychlorinated biphenyls (PCB 28, PCB 52, PCB 101, PCB 138, PCB 153, PCB 180, PCB sum) of the compound feed produced in unit B, they were below the detection limit (2 ppb) for the analyzes performed in both years of study.

– the **biological hazards** associated with the production of compound feed from units studyed were analyzed and controlled during 2019 and 2020.

In order to determine the biological hazards of raw materials and compound feed, mycological and bacteriological analyzes were performed. In **unit A**, the results of mycological analyzes performed to determine the total number of fungi in the raw materials showed that the proportion of isolation of the genus *Aspergillus* was predominant in corn and wheat grains (over 75%) and in soybean and sunflower meal (over 65%). Also, in the case of mycologically analyzed compound feeds, the genus *Aspergillus* was identified in the majority (between 34.6% and 53.3%).

Exceedances of the maximum permissible limits were found in terms of the total number of fungi in the analyzed raw materials; thus, the maximum value identified for maize grain was 8000 cfu/g, for wheat grain 6000 cfu/g and for sunflower meal 2000 cfu/g. For compound feed, there was no exceeding of the maximum limit allowed by law.

All results of microbiological analyzes performed for raw materials and compound feed to determine contamination with *Salmonella* spp., *E. coli* and *Clostridium perfringens*, were negative.

In the case of raw materials and compound feed from **unit B**, all analyzes performed to determine *Salmonella* spp. contamination were negative; also, sanitation tests for the determination of *Salmonella* spp. in the processing environment had negative results.

Following the research carried out and the results presented on the production of compound feed in relation on food safety, we make some **recommendations**:

– permanent monitoring of raw materials and compound feed in order to ensure the safe provision of food of animal origin for the final consumer;

– at the legislative level, the establishment of regulated microbiological limits for yeasts and molds, due to the fact that a legislative void was created following the abrogation of Order no. 249/2003;

– the establishment by the competent authorities of maximum permitted levels for co-occurrence of mycotoxins in raw materials of plant origin and compound feed.