ABSTRACT

The turkey poultry sector is one of the most dynamic poultry sectors, even if it has not yet reached the superior level of broiler breeding sector. This dynamics shows a trend of expansion in new markets where the consumption of turkey meat was until very recently at very low levels, but the need to have a viable alternative to chicken and the superior nutritional qualities of turkey meat were the main engine of expansion of this animal livestock branch.

In the growth of turkey broiler and, in general, in the intensive livestock breeding for meat production, respiratory diseases have always been a continuous and increasingly pressing challenge, taking into account the advance of genetics in the creation of hybrids to which the rapid growing was the key desideratum. Infectious turkey rhinotracheitis virus (TRT) and *Ornithobacterium rhinotracheale* are two of the most important respiratory infectious agents in the infectious turkey pathology with strong sanitary-veterinary and economic-financial implications.

Avian metapneumovirus affects the upper respiratory tract in turkeys and causes turkey infectious rhinotracheitis disease (TRT), and in other species it is involved in the occurrence of a disease with polyfactorial etiology called "swollen head syndrome" in the cicken broiler, was reported for the first time In South Africa in the 1970s, and a decade later he would also appear in poultry holdings in Europe. Since then, the presence of TRT has been reported worldwide, including the United States of America.

This avian paramyxovirus include four subtypes (A, B, C and D), and in present the subtype B is isolated and identified most often at European level, which is why the related industries, such as the one producing the immunological preparations, are using more frequently the same subtype as the vaccine substrate. Infectious turkey rhinotracheitis (TRT) have a particular importance for the turkey broiler breeding industry, mainly due to the very high losses it causes both directly through mortality and the lack of technological and economic indicators, as well as indirectly through increased costs for specific immunizations and applied drug treatments. This avian metapneumovirus has a very rapid spread and, most of the time, it is only the triggering factor of the respiratory syndrome in the turkey, it is the one that prepares the ground for subsequent engraftment of synergistic respiratory infections.

Infections produced by *Ornithobacterium rhinotracheale* have been observed since 1991, but the first characterization was performed only two years later, and after a very short period of time, the presence of this bacterium has been reported worldwide in almost all bird species. At present, 18 serotypes (identified from A to R) have been characterized and identified, but the serotypes A, B, D and E were most often isolated in turkeys. Due to this very large variability to date, there are not yet commercial vaccines constructions which can provide satisfactory results, which is why the prevention and control of this morbid entity in turkeys are

to limitated to reduce the possible losses using the autogenous vaccines and the introduction of appropriate medical treatment as soon as it has been installed at the unit of growth acute respiratory syndrome.

In order to make a fair evaluations of infections caused by infectious rhinotracheitis virus of turkeys and by *Ornithobacterium rhinotracheale*, the plan of measures to be implemented will be one that will take into account all the harmful biotic factors involved in the occurrence and the development of respiratory diseases group, to achieve the highest effectiveness of the action plan undertaken.

The doctoral thesis entitled "***Researches regarding themethodology of diagnosis, prevention and control in somerespiratory diseases in turkeys intensively reared***" aimed primarily to identify the main infectious agents in the respiratory pathology of hybrids which shows a particular impact on this industry, but also quantifies the impact and assesses the effectiveness of the main diagnostic, prophylaxis and combating measures implemented at the level of breeding and fattening units that participated in research in 2010-2016.

This doctoral thesis covers a total of 199 pages, 46 tables and 129 figures and, according to the norms in force, it consists in two main parts, namely the first part entitled "***The Current Stage of Knowledge***" which cover 29 pages and five figures, and the second part, entitled "***Personal Contributions***", spreaded over 135 pages, 46 tables and 124 figures.

The "Current state of knowledge" consists on two chapters summarizing information from the literature referring to the subject of the PhD thesis and subsequently used for the interpretation and comparison of the data obtained in the "***Personal Contributions***" part.

The first chapter, named "***The concepts of anatomy and physiology of respiratory apparatus in turkeys (Meleagridis gallopavo)***", is structured in two subchapters and synthesizes information from the literature regarding anatomy and physiology of the respiratory apparatus - breathing process in birds and as well as important information concerning the specific immune system of the respiratory immunity.

In the last chapter, which end the bibliographic study part in the field of researched doctoral research, there are exposed by thorough scientific documentation and latest information on the main infectious diseases with respiratory tropism encountered in turkeys intensive breeding system. Between the respiratory viral infections of turkey hybrids raised in the intensive system for meat production, these researches were focused on infectious turkey rhinotracheitis (TRT), which was extensively treated in the first subchapter of this chapter of the doctoral thesis. In the second subchapter, bacterial infections of turkeys were extensively treated regarding infections caused by *Ornithobacterium rhinotracheale* and its implications in infectious respiratory pathology for this species.

The "***Personal Contributions***" section debuts with Chapter 3 entitled "***The organizational and institutional framework in which the research was conducted***". In this chapter is described firstly the natural framework with the geographical (administrative-territorial) location of the research site, thus are individualized all the seven livestock breeding and fattening units of the turkeys, respectively Agro Nariman, Cărpiniş, Ferma 6, Romad Otis, Farm 1, Farm 2 and Farm 5 located in the districts of Braşov and Mureş counties, as well as the number of series distributed on each farm in 2010-2016 period of time. Then, we presented the organizational and instutional framework were the research was carried out, briefly presentation of all six research and diagnostic units involved in this academic work.

In Chapter 4 was presented the main purpose of the researches carried out, as well as

the four main objectives pursued for the realization of the proposed scientific goal.

In the following chapter, entitled "***Epidemiological Study of Respiratory Infections with TRT and ORT in Intensive Turkeys***", a retrospective epidemiological investigation was carried out covering the period 2010-2016 regarding the epidemiological mortality indicator on all farms taken starting on first day after population until delivery to the slaughterhouse. This investigation targeted 82 series of breeding and fattening turkey hybrids representing 8659750 individuals. The mortality rate recorded in the growing and fattening period studied in this research registerred a average of 7.61%, of which 5.74% in hens (extreme values of 1.2% and 23.8%), and 9.71% in turkeys (extreme values of 3.37% and 35.13%).

The highest mortality rate for hens and turkeys was recorded in 2012 (14.34%), while the lowest mortality was recorded in 2011 (5.25%). Depending on the type of meat hybrid used as a genetic material in the hens, the lowest median mortality was recorded in the BUT 6 hybrid (3.12%), while in turkeys the lowest mortality was recorded in the Grade hybrid Maker (7.81%). In addition, it was found that when a single source of genetic material and a single hybrid was used, the registered mortality indicator had an average of 6.18% (3.67% in hens and 8.69% in turkeys) in while the same epidemiological indicator recorded an average had a value of 9.34% (8.09% for hens and 10.59% for turkeys) when multiple types of broilers were used from multiple sources.

When were analyzed the age range correlated with the mortality indicator, it was shown that the period immediately after the population (the first 21 days of life) represents the age range with the highest incidence of the epidemiological mortality indicator, regardless of sex, type of broiler or country of origin, and in addition, the range of 8-12 weeks have a particular importance in terms of the economic implications of age-related mortality.

"***Clinical and anatomopathological investigations of respiratory infections caused by Infectious rhinotracheitis of turkeys (TRT) and rhinotracheal orthobacterium (ORT) infections in turkeys***" represent the chapter 6 of the Ph.D. thesis where have shown that respiratory diseases in turkeys raised in the industrial system are difficult to be differentiated only on the basis of the clinical and lesional picture, and that requiring laboratory investigations for certainty. Even if both clinical signs and lesional pictures do not present disease-specific elements, the results provided by them may guide the on-going diagnostic protocol, which have a vital importance for the practitioner who can apply the most effective measures as soon as possible, thus minimizing possible losses.

The most complex research in this paper is presented in Chapter 7 entitled "***Implementation of a specific prophylaxis methodology for respiratory infections caused by TRT and ORT in turkeys***" and consisted in implementing the most effective methodology by which the most appropriate prophylaxis protocol for the reperfusion injuries involving TRT and ORT, but also is evaluated the effectiveness of the implemented measures, and is make a prospective qualitative analysis using serological, bacteriological and molecular biology methods.

Practically, the farms were first grouped into three main categories according to several factors: farms with low microbial pressure (Agro Nariman, Carpiniș and Farm 6), medium-pressure farms (Romad Otis) and farms with increased microbial pressure (Farm 1, Farm 2 and Farm 5). Then, according to this catalog, the most appropriate TRT and ORT specific immunization protocol was implemented and the efficacy of the measures undertaken was assessed. Unconcerned of the exogenous microbial load existent at each zootechnical unit

participating in the study, there were three serological screenings for each farm, each serological screening being assigned a series of growth and fattening of turkey hybrids.

In the case of farms with low exogenous microbial pressure (Agro Nariman, Cărpiniş and Farm 6), no specific immunization for ORT was performed, but specific immunization for TRT (two doses at screening 1, respectively five immunizations at the other screenings) was performed and the results obtained showed that the immunological protocol used had favorable results, the measures being considered to be sufficient under the given conditions.

At Romad Otis after immunizing for the TRT in the first series using a vaccination schedule that included two vaccinations and did not specifically immunize against ORT and the results were unfavorable, considerable losses being recorded, so it was decided that starting from the next series (screening 2) to carry out immunization for the first time in this zootechnical breeding and fattening unit of turkeys against ORT (two autologous vaccine administrations using isolated outbreak strains from the previous series) supplemented with multiple immunizations for TRT. The comparative results showed unequivocally the effectiveness of the specific prophylactic protocol implemented.

In the farms with high microbial pressure, a specific immunoprofilactic protocol consisted of multiple vaccinations for TRT (two, five or even six administrations) as well as vaccination for ORT (two administrations of autovaccin), which resulted in to an improvement of the technical and sanitary indicators and implicitly of the economic and financial ones compared to the previous series, thus demonstrating the effectiveness of the measures taken.

Research has shown that the synergism TRT-ORT, TRT-ORT and other bacteria is present, as a rule TRT plays the role of trigger factor, followed by ORT as well as other bacteria such as *Bordetella bronchiseptica*, *Riemerella anatipestifer*, *Pseudomonas aeroginosa*, *Escherichia coli*, etc. which have been identified using laboratory methods.

Regarding immunization against infectious turkey rhinotracheitis virus (TRT), research has shown that the degree of resistance to this disease is directly proportional to the number of specific immunizations noted phenomenon when compare the immunized series twice with those immunized five times during life.

The results obtained following the quantitative real time PCR test on turkey infectious rhinotracheitis virus (TRT) led to the identification of subtypes A and/or B in all zootechnical sites participating in the study.

The results provided by mass spectrometry using MALDI TOF, corroborated with those provided by quantitative gene amplification by real time PCR, resulted in the identification of *Ornithobacterium rhinotracheale* bacteria, serotypes A, B, C, E or I/J in all the studied farms.

The results obtained, irrespective of the type of zootechnical farm, indicated that the implemented protocol was constantly the most viable option, bringing an improvement of the indicators pursued, and the specific immunization for both TRT and ORT played a decisive role in strengthening local immunity, Even if they were unable to provide complete immunity against wild strains.

In addition to support the measures implemented in the previous chapter, it has proved to be very necessary and useful a much more stringent analysis that was presented in Chapter 8 "***The Study on the economic impact of applied strategies***" where it is also argued economic point of view the effectiveness of the immunoprofilactic program implemented in the growing and fattening of intensive turkey hybrids in Romania.

In the case of farms located on the Codlea poultry platform (Farm 1, 2 and 5), where microbial pressure is increased, there is a cost of immunizations ranging from 39% to 41%, and for Romad Otis where the microbial pressure is at medium level, there is a cost of immunizations of 37%. In contrast, for low-pressure farms (Cărpiniş, Agro Nariman and Farm 6), immunization costs range from 14% to 20% of the total budget allocated to sanitary veterinary actions, which represents only 5.90% of the total expenditure registered with a series .

The effectiveness of immunoprofilactic measures implemented regard to TRT and ORT are both direct expenditure on vaccines that account for only 1.68% of the total costs of a series and the positive impact of these measures assessed by decreasing mortality at critical ages, decreasing the number of reformed in the slaughterhouse, reducing the costs of neutralization and, implicitly, increasing the quantity of meat delivered for sale but also its superior quality.

As in any scientific work, the last chapter is "*Conclusions and Recommendations*" in which there are synthetically presented 18 conclusions and 16 recommendations that come out of this paper.