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AGRICULTURAL SCIENCES AND VETERINARY MEDICINE  
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THE FACULTY OF VETERINARY MEDICINE  
DOMAIN: VETERINARY MEDICINE  
SPECIALIZATION: VETERINARY TECHNOLOGY AND EXPERTISE**

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**PhD DISSERTATION**

**Assessment of heavy metal and  
nitrate/nitrite residues in the plant,  
part of food chain and their risk for  
consumers**

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## **ABSTRACT**

PhD thesis with the topic **„ASSESSMENT OF HEAVY METAL AND NITRATE/NITRITE RESIDUES IN THE PLANT, PART OF FOOD CHAIN AND THEIR RISK FOR CONSUMERS”**, is structured into two distinct parts, in 7 chapters: bibliographic study and personal researches, covers 249 pages, 92 tables and 58 figures.

The first part - Bibliographic study - is systematized in 2 chapters over 69 pages, illustrated by 4 figures and 33 tables in which the informations from bibliographic references is related to:

- sources of contamination with heavy metals (Pb, Cd, Cu, Zn) and their incidence in vegetal origin feeds; sources of contamination with nitrates/nitrites, their incidence and factors that contribute to the accumulation in vegetal origin feeds;
- heavy metal and nitrate/nitrite effects on production and animal health;
- legislative measures of quality, safety and chemical risk assesment on food and feed.

Part two - Personal researches - is systematized in 5 chapters over 144 pages, illustrated by 54 figures and 59 tables. In Chapter III are presented: researches objectives, the motivation of theme, working locations, indicators considered for the study, researches organization, material and research methods. Chapters IV, V, VI present research results on the identification, quantitative and risk assessment of Pb, Cd, Cu, Zn and nitrate/nitrite residues from some vegetal feeds and from feed and mixed feeds rations, accompanied by discussion of results and partial conclusions. The paper ends with Chapter VII - General conclusions and recommendations. Development work is supported by 285 bibliographic sources.

The main objectives of the undertaken researches in 2006÷2008 years was to identify and assess the level accumulation of heavy metal and nitrate/nitrite residues in vegetal origin feed samples, feed rations, mixed feeds samples and their risk for consumers. For researches, were taken 175 samples of 12 varieties of vegetable feeds from the unit „A”, and 105 samples of 7 varieties of vegetable feeds from the unit „B”. The units located in Iasi County, have different production profiles: Unit „A” is profiled on growth of dairy cattle and Unit „B” on broiler chickens increase. Working techniques (collection, training and preparation of laboratory samples), determinate dry matter content, heavy metals content (atomic absorption

spectrometry) and nitrates/nitrites content (spectrofotocolorimetrie) methods, were conducted with standard rules respect from Romania and EU legislation.

The data of laboratory analysis undertaken to identify and assess heavy metals throughout the research period 2006÷2008, shown the presence of these elements in all vegetal origin feed samples, with the following values:

#### Unit „A”

- average content of Pb was  $1,32\pm 0,03$  mg/kg, with limits of  $0,11\pm 0,01$  mg/kg in wheat bran samples and of  $3,86\pm 0,11$  mg/kg in sunflower samples, in 2008 year. Pb accumulation in vegetal origin feed samples, were decreased with 32,53%, from  $1,66\pm 0,04$  mg/kg in 2006 year, to  $1,12\pm 0,04$  mg/kg in 2008 year; average content of Cd was  $0,092\pm 0,003$  mg/kg, with limits of  $0,016\pm 0,001$  mg/kg in wheat bran samples, in 2006 year and of  $0,208\pm 0,007$  mg/kg at alfalfa hay samples, in 2008 year. Cd residues in vegetable samples were decreased from  $0,107\pm 0,003$  mg/kg in 2006 year, to  $0,076\pm 0,001$  mg/kg in 2007 year, with 28,97% and an increase with 23,68% in 2008 year compared to 2007, to  $0,094\pm 0,004$  mg/kg;

- average content of Cu was  $3,38\pm 0,09$  mg/kg, with values ranging from  $0,30\pm 0,01$  mg/kg in brewery dregs samples, in 2007 year, to  $8,48\pm 0,41$  mg/kg in vetch samples, in 2008 year. The average concentration of Cu, in the feed samples analyzed, decreased in 2007 year compared to 2006, with 14,46%, from  $2,49\pm 0,06$  mg/kg to  $2,13\pm 0,01$  mg/kg and an increase of 2,61 times in 2008 compared to 2007 year, to  $5,54\pm 0,20$  mg/kg value;

- average content of Zn was  $4,042\pm 0,157$  mg/kg, with values ranging from  $0,089\pm 0,001$  mg/kg in wheat bran samples, in 2007 year, to  $12,828\pm 0,775$  mg/kg at Sudan grass samples, in 2008 year. Zn content of feed samples, showed a significant increase of 5,83 times, from  $1,545\pm 0,023$  mg/kg in 2006 year, to a value of  $9,013\pm 0,443$  mg/kg in 2008 year;

#### Unit „B”

- average content of Pb in raw materials was  $0,91\pm 0,03$  mg/kg, with limits of  $0,18\pm 0,01$  mg/kg in wheat samples, in 2007 year and of  $2,03\pm 0,04$  mg/kg in wheat samples, in 2006 year; the accumulation of Pb in mixed feeds was  $0,84\pm 0,02$  mg/kg, with limits of  $0,43\pm 0,01$  mg/kg in 2007 year and of  $1,47\pm 0,02$  mg/kg in 2006 at finisher broiler mixed feed. Average content of Pb, in raw materials, decreased in 2006 year, from  $1,33\pm 0,04$  mg/kg to  $0,67\pm 0,03$  mg/kg in 2008, with 49,62% and in mixed feeds, the decrease was 48,36%, from  $1,22\pm 0,03$  mg/kg in 2006 year to  $0,63\pm 0,02$  mg/kg in 2008 year;

- average content of Cd in raw materials was  $0,105\pm 0,002$  mg/kg, with limits of  $0,024\pm 0,001$  mg/kg in full fat samples, in 2006 and of  $0,180\pm 0,001$  mg/kg in soybean meal and full fat soybean samples, in 2007 year and in mixed feeds samples was  $0,091\pm 0,001$

mg/kg, with limits of  $0,060\pm 0,02$  mg/kg and of  $0,115\pm 0,001$  mg/kg in grower broiler mixed feed from 2006 year, respectively 2008 year. Average content of Cd was decreased in 2008 compared to 2006 year, at raw materials with 1,1%, from  $0,091\pm 0,002$  mg/kg to  $0,090\pm 0,003$  mg/kg and at mixed feeds with 19,15%, from  $0,094\pm 0,002$  mg/kg to  $0,076\pm 0,002$  mg/kg;

- average content of Cu was  $9,40\pm 0,36$  mg/kg, with limits of  $2,96\pm 0,12$  mg/kg in maize samples, in 2006 and  $16,64\pm 0,51$  mg/kg in full fat soybean samples, in 2008 year, for raw materials and in mixed feeds, was of  $6,93\pm 0,24$  mg/kg, with limits  $6,18\pm 0,22$  mg/kg in grower broiler mixed feed, in 2006 and of  $7,84\pm 0,29$  mg/kg in finisher broiler mixed feed, in 2008; at raw materials, Cu increased in 2008 compared to 2006, with 12,11%, from  $9,08\pm 0,44$  mg/kg to  $10,18\pm 0,37$  mg/kg and at mixed feeds, with 13,91% from  $6,54\pm 0,22$  mg/kg at  $7,45\pm 0,27$  mg/kg;

- average content of Zn was  $17,721\pm 0,940$  mg/kg, with limits of  $5,236\pm 0,253$  mg/kg in maize samples and of  $35,352\pm 0,877$  mg/kg in full fat soybean samples, in 2007 year, at raw materials; average content of Zn in mixed feeds, was  $38,146\pm 0,762$  mg/kg, with limits of  $29,993\pm 0,832$  mg/kg in finisher broiler mixed feed, in 2006 year and of  $43,483\pm 0,639$  mg/kg in grower broiler mixed feed, in 2007 year; average content of Zn increased in 2008 compared to 2006, at raw materials, with 22,43%, from  $15,516\pm 0,499$  mg/kg to  $18,996\pm 0,607$  mg/kg and at mixed feeds with 20,66%, from  $34,101\pm 0,812$  mg/kg to  $41,145\pm 0,707$  mg/kg.

The same vegetal origin feed samples were used for the determination of nitrates/nitrites content in 2006÷2008 years. In all samples from the two units „A” and „B”, were found different levels of nitrates/nitrites content, with the following values:

#### Unit „A”

- average content of nitrates was  $47,29\pm 0,58$  mg/kg with limits of  $6,36\pm 0,25$  mg/kg in sunflower meal samples, in 2008 year and of  $210,94\pm 1,81$  mg/kg in vetch samples, in 2006 year; the average content of nitrites was  $4,68\pm 0,11$  mg/kg with limits of  $0,11\pm 0,01$  mg/kg in sunflower meal samples, in 2008 year and of  $11,09\pm 0,15$  mg/kg in alfalfa samples, in 2007 year. Average content of nitrates level has decreased in 2008 compared to 2006, with 10,23%, from  $49,76\pm 0,56$  mg/kg to  $44,67\pm 0,61$  mg/kg and average content of nitrites has decreased with 31,89%, from  $5,08\pm 0,11$  mg/kg to  $3,46\pm 0,14$  mg/kg;

- in all three series of researches, the highest quantitative values of nitrates, were recorded in the green foddes with average content between  $92,00\pm 1,02$  mg/kg and  $111,33\pm 1,17$  mg/kg limits, compared with the average values of fodder silage registered between  $40,59\pm 0,51$  mg/kg and  $43,67\pm 0,33$  mg/kg limits and in hay fodders between  $22,67\pm 0,23$  mg/kg and  $23,71\pm 0,49$  mg/kg limits. The lowest concentration of  $\text{NO}_3^-$  was found

in cereals and industrial products with average values between  $8,84\pm 0,28$  mg/kg and  $12,60\pm 0,26$  mg/kg limits. The concentration of  $\text{NO}_3^-$  in green fodders were higher than fodder silage with 58,56% and with 77,19% compared with hay fodders and with 89,46% higher than the average concentration founded in cereals and industrial products.

#### Unit „B”

- the average content of nitrates, in raw materials, was  $12,48\pm 0,38$  mg/kg, with limits of  $9,49\pm 0,52$  mg/kg in maize samples, in 2007 and of  $19,47\pm 0,46$  mg/kg in wheat samples, in 2006 year and in broiler mixed feeds, the value was  $14,30\pm 0,28$  mg/kg, with limits of  $11,90\pm 0,26$  mg/kg in finisher broiler mixed feed, in 2008 and of  $16,91\pm 0,18$  mg/kg in starter broiler mixed feed, in 2006 year;

- the average content of nitrites, in raw materials, was  $3,39\pm 0,10$  mg/kg, with limits of  $1,10\pm 0,02$  mg/kg in full fat soybean samples, in 2008 and of  $10,31\pm 0,47$  mg/kg in wheat samples, in 2007 year and in broiler mixed feeds was  $3,47\pm 0,05$  mg/kg, with limits of  $1,75\pm 0,05$  mg/kg in grower broiler mixed feed, in 2008 year and of  $5,13\pm 0,07$  mg/kg in grower broiler mixed feed, in 2007 year; average content of  $\text{Na NO}_2$  was of  $4,58\pm 0,08$  mg/kg, with limits of  $2,31\pm 0,07$  mg/kg, in 2008 year and of  $6,77\pm 0,09$  mg/kg, in 2007 year, at grower broiler mixed feed;

- the nitrates content in raw materials decreased in 2008 compared to 2006, with 11,77%, from  $13,17\pm 0,35$  mg/kg to  $11,62\pm 0,29$  mg/kg and at broiler mixed feeds with 22,71%, from  $15,81\pm 0,23$  mg/kg to  $12,22\pm 0,17$  mg/kg;

- the nitrites content in raw materials decreased in 2008 compared to 2006, with 2,18 times, from  $3,99\pm 0,06$  mg/kg to  $1,83\pm 0,07$  mg/kg and at broiler mixed feeds with 2,13 times, from  $4,18\pm 0,05$  mg/kg to  $1,96\pm 0,06$  mg/kg; the nitrites content expressed as sodium nitrite, calculated for broiler mixed feeds, decreased in 2008 compared to 2006, with 2,13 times, from  $5,51\pm 0,07$  mg/kg to  $2,58\pm 0,08$  mg/kg.

With analytical data obtained, was calculated intake of heavy metals and nitrates/nitrites in feed winter and summer rations for lactating cows of 600 kg and 20 kg average milk production and in broiler mixed feeds and the risk exposure of consumers.

Average quantitation of feeds rations in 2006÷2008 years was  $24,80\pm 0,37$  kg. The values of average intake of heavy metals and nitrates/nitrites were:

- the average intake of Pb was estimated at  $32,23\pm 3,59$  mg/ration/head and  $1,29\pm 0,14$  mg/kg ration and with an exposure between  $0,03\div 0,09$  mg/kg b.w. (body weight) limits; the average intake of Cd was estimated at  $2,474\pm 0,360$  mg/ration/head and  $0,100\pm 0,015$  mg/kg ration and with an exposure between  $0,002\div 0,006$  mg/kg b.w. limits;

- the average intake of Cu was estimated at  $82,18 \pm 19,90$  mg/ration/head and  $3,76 \pm 1,00$  mg/kg ration and with an exposure between  $0,07 \div 0,24$  mg/kg b.w. limits; the average intake of Zn was estimated at  $96,181 \pm 29,493$  mg/ration/head and  $4,416 \pm 1,940$  mg/kg ration and with an exposure between  $0,043 \div 0,332$  mg/kg b.w. limits;

- the average intake of nitrates was estimated at  $1129,12 \pm 19,5$  mg/ration/head and  $51,22 \pm 1,01$  mg/kg ration and with an exposure between  $1,19 \div 3,15$  mg/kg b.w. limits; the average intake of nitrites was estimated at  $119,20 \pm 14,63$  mg/ration/head and  $5,31 \pm 0,46$  mg/kg ration, with an exposure between  $0,14 \div 0,32$  mg/kg b.w. limits;

Values obtained by calculated the average intake of heavy metals and nitrates/nitrites by the 4075 g broiler mixed feeds, in 42 days, in 2006÷2008 years, were:

- the average intake of Pb was  $0,83 \pm 0,21$  mg/kg mixed feed, with a exposure between  $1,04 \div 2,58$  mg/kg b.w. limits; the average intake of Cd was  $0,095 \pm 0,008$  mg/kg mixed feed and with a exposure between  $0,140 \div 0,212$  mg/kg b.w. limits;

- the average intake of Cu was  $6,40 \pm 0,20$  mg/kg mixed feed, with a exposure between  $11,38 \div 13,07$  mg/kg b.w. limits; the average intake of Zn was  $33,225 \pm 1,58$  mg/kg mixed feed and with a exposure between  $55,742 \div 68,961$  mg/kg b.w. limits;

- the average intake of nitrates was  $12,36 \pm 0,62$  mg/kg mixed feed and with a exposure between  $20,62 \div 25,78$  mg/kg b.w. limits; the average intake of nitrites was  $3,33 \pm 0,61$  mg/kg mixed feed and with a exposure between  $3,32 \div 7,93$  mg/kg b.w. limits;

- the average intake of nitrites content expressed as sodium nitrite, was  $4,398 \pm 10,46$  mg/kg mixed feed and with a exposure between  $4,38 \div 10,46$  mg/kg b.w. limits.

The results obtained, indicate the presence of heavy metal and nitrate/nitrite residues in all vegetable feed samples. The results were used to calculate the exposure to those pollutants by their intake once with feed rations or mixed feeds. Analytical data, can be indicators of the status of soil pollution which is reflected by the contamination level of feeds from the studied area and from a determined period.

The results of the investigation form a database which complete and update the existing one; those results can be an argument for continued monitoring of pollutants, activity that to ensure for a long period, safety and quality of vegetal feeds. The values results of assessment of heavy metal and nitrate/nitrite content in vegetal origin feed samples, from unit „A” și „B”, were under the maximal limits admitted, established by the national and international legislation. All of 280 feeds samples are adequate and doesn't endanger the safety of production and animal health.