

ABSTRACT

The Doctoral Thesis entitled **RESEARCHES REGARDING COPPER AND ZINC DEFICIENCY IN CATTLE** has the character of originality by assessing the impact of copper and zinc deficiency on the performance of health, production and reproduction in Romanian Black Pie dairy cattle, comparative analysis of the copper and zinc status of Holstein and Brown Swiss cattle in advanced gestation and the investigation of the copper, zinc, selenium, iron status, hematologic profile and to establish the correlations between Montbeliarde and Charolaise cattle and their calves. The originality of the thesis is shown from the analysis of copper and zinc status in Romanian Black Pie dairy calves and their correlations with hematological and biochemical profiles, but also by establishing where the use of physiological parameters in adult cattle is inappropriate for calves.

The thesis includes a total of 267 pages and is structured in two parts.

The first part - General Considerations consists of 49 pages, being organised in four chapters which presents selected data from bibliographic sources of literature on copper and zinc in cattle and interrelations of these oligo-minerals.

Part two – Personal Contributions cover a total of 165 pages and contains 8 chapters, that presents the purpose and importance of researches, material and methods, results, related discussions and general conclusions emerged from researches.

Presented data are supported by 7 tables and 6 figures inserted into the General Considerations part, 17 tables and 67 figures inserted in the Personal Contributions part. Bibliographic list totals 260 titles of local and international literature and Internet files with specialized content. The thesis has data published in scientific works supported in Scientific Symposia with international participation, in Romanian Journal of Veterinary Medicine and in Agronomical Research in Moldavia journal.

The importance of the chosen theme comes from the fact that deficiencies in oligo-elements negatively affects health, production and reproduction in cattle. Mineral supplementation of rations in cattle is important not only for animals but also for farmers who could benefit a greater productivity of his cattle, and a better financial gain if the trace element status in animals would be appropriate. Once the animals are deficiented, their products (meat, milk) are also deficiented. Meat and milk are consumed by people who needs a sufficient intake of oligo-elements to their health. There are studies that clearly demonstrated the positive impact of mineral supplementation on health, so adding oligo-elements in animals has positive effects on humans.

The research aim was to assess the copper and zinc status in different breeds (Romanian Black Pie, Holstein, Brown Swiss, Charolaise and Montbeliarde) cattle, age (calves, adult) and physiological status (lactation, advanced gestation). To achieve this goal we covered the following objectives:

- ✓ Evaluation of copper and zinc status according administration of these trace elements in the form of sulphates and correlation with their performance of health, production and reproduction in Romanian Black Pie dairy cattle.
- ✓ Analysis of the copper and zinc status in Holstein and Brown Swiss cattle in advanced gestation and their correlations with hematological and biochemical profiles.
- ✓ Analysis of copper and zinc status in Romanian Black Pie dairy calves and their correlations with hematological and biochemical profiles.
- ✓ Recording of hematological and biochemical profiles changes in calves during the first month of life and setting where the use of physiological parameters in adult cattle is inappropriate for calves.
- ✓ Investigation of copper, zinc, selenium, iron status, haematological profile and their correlations between Montbeliarde cattle and their calves.
- ✓ Investigation of copper, zinc, selenium, iron status, haematological profile and their correlations between Charolaise cattle and their calves.

To achieve the study, researches were conducted in four cattle farms in Romania and France. The laboratory investigations were conducted in laboratories of Medical Clinic, Physiology, Physiopathology and Histopathology of the Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu Brad", Chemistry laboratories of the University Alexandru Ioan Cuza Iassy and Dorna Lactate SA Floreni laboratory, Vatra Dornei.

Laboratory studies of cattle farms in France, were made in laboratories of Pathology Clinic of cattle and Toxicology of VetAgro Sup Campus veterinaire de Lyon, France. These laboratories, together with associated clinics and cattle farms in northeastern Romania and regions of France have provided the necessary to achieve the objectives.

In chapter 6, were conducted researches regarding the copper and zinc status according administration of these trace elements in the form of sulphates (2 versus 8,5 ppm copper and 9 versus 42 ppm zinc) and their correlations with performance of health, production and reproduction in Romanian Black Pie dairy cattle, on four groups of 10 cattle, over a period of 20 weeks. Cattle of the Copper group received 2 g CuSO_4 /cattle/weeks, those of Zinc group received 10 g of ZnSO_4 and those of Copper-Zinc group 2 g CuSO_4 and 10 g ZnSO_4 . Resulting from research conducted in the early observations that 60% of cattle were in copper and zinc

deficiency. It was noted that cattle that have received Cu and/or Zn had a higher body weight than those of Control group, differences were not statistically assured. Rumen refilling score did not show significant changes between the first and last week except cattle of Copper group, which was found a significant improvement ($P < 0.05$) during the four weeks that have received grass. Also during feeding with grass, the values of undigested faecal fraction score in cattle that received mineral supplements, were lower statistically significant ($P < 0.05$) than in the Control group. Score from the hocks lesions recorded higher values in cattle from the Control group compared to groups which received mineral supplements, the differences being statistically significant ($P < 0.05$). Histopathological aspects of the cattle skin with periorbital acromotrichie (glasses) showed depigmentation of the skin, agglutination of melanocytes in the dermis, hair follicle degeneration and poorly pigmented melanocytes in the sheaths of hair follicle. A significantly higher milk production ($P < 0.05$) in cattle of Zinc group at t_2 (day 56 of study) and t_3 (day 84 of study) compared with Zinc-Copper group. Pearson test showed a positive correlation ($P < 0.01$) between plasma zinc and milk production at t_3 , but was no correlation between plasma copper and milk production. Protein and fat of milk at t_5 (day 140 of study) were lower in Control group than supplemented groups, but the differences were not statistically significant. Pearson test showed a positive correlation ($P < 0.01$) between plasma zinc and fat of milk, but was no correlation between plasma copper and fat milk. During the 20 weeks of study, the interval first insemination - fertilized insemination ranged from 38 days in cattle of Copper group and 66 days in Control group. Copper deficiency is not sufficient to cause hypochromic and microcytic anemia. Total leukocyte values in the 4 groups of cattle were classified as physiological limits, but the total number of leukocytes was higher in Control group with no statistically significant differences ($P > 0.05$). Ration supplementation in copper and zinc sulphate, allowed an increase in the concentrations of these trace elements in plasma. The values of copper plasma in the four groups recorded a significant development but since the second month of extra rations, cattle reached the normal range of cupremiei. Note that since t_4 (day 112 of study), it was a significant difference of copper plasma between Copper group, and Control ($P < 0.01$) and Zinc ($P < 0.05$) groups on the other hand. During the entire study, was noted a substantial change in plasma zinc within each group ($P < 0.01$). The supplemented groups with $ZnSO_4$ reached the normal parameters of plasma after a month of administration of $ZnSO_4$, while other groups, two months later. Pearson statistical test showed no correlation between changes of plasma copper and plasma zinc in cattle of four groups.

In chapter 7 were made researches regarding the copper and zinc status and their correlations with hematological and biochemical profiles in Holstein and Brown Swiss cattle in advanced gestation, divided into two groups of 10 cattle each (group Holstein and group Brown

Swiss). Analyzing the results, it appears that the average values of erythrocyte indices were higher in Brown Swiss than Holstein cattle, the differences were not statistically assured. Ceruloplasmin of plasma and serum alkaline phosphatase were determined to assess the Cu and Zn status. Pearson test showed no statistically significant correlations between values of red blood cell and plasma ceruloplasmin. Mean values of alkaline phosphatase ranged statistically insignificant in Holstein and Brown Swiss cattle, maintaining in physiological parameters. Pearson test showed no correlation between changes in alkaline phosphatase values and the leukograms in cattle. In all 20 cattle of study, ceruloplasmin had values less than physiological lower limit, indicating the evolution of copper deficiency. Ceruloplasmin in Brown Swiss cattle (54.97 ± 15.62 mg/L) was statistically significant ($P < 0.05$) higher than in Holstein cattle (5.18 ± 40.60 mg/L). Copper deficiency in Holstein and Brown Swiss cattle in advanced pregnancy, was not sufficient to cause a microcytic and hypochromic anemia. Glucose values in both breeds have been located to the lower physiological limit, and this must to be taken into account in energy metabolism, with β -OH-butyrate and cholesterol levels. We found no statistically significant correlations between ceruloplasmin and alkaline phosphatase concentrations, neither between serum calcium and alkaline phosphatase.

In chapter 8 were made researches regarding the copper and zinc status in Romanian Black Pie dairy calves (BNR) and their correlations with hematological and biochemical parameters, indicating where the use of physiological parameters in adult cattle is inappropriate for calves. Observations were made on 40 clinically healthy calves BNR divided into eight groups according to age: group 1 = one day, group 2 = 2 days, group 3 = 3-6 days group 4 = 8 days, group 5 = 11 days, group 6 = 16 to 18 days, group 7 = 24 days, group 8 = 28 to 29 days. Plasma ceruloplasmin and serum alkaline phosphatase were determined to assess the Cu and Zn status. The investigations made clear that the red blood cell indices in calves of the study a tendency to decrease until the end of neonatal period. Lowest values of Hb and Ht as were observed in calves aged 11 days coincides with the change of milk. Pearson test showed statistically significant positive correlation ($P = 0.001$) in the evolution of ceruloplasmin on the one hand and erythrocyte indices on the other hand. Pearson statistical test showed no correlation between changes in plasma ceruloplasmin and erythrocyte constants. Calf age had a statistically significant effect ($P < 0.05$) on platelets, the lowest values were observed in calves aged two days, while the highest values were in calves aged 11 and 18 days. Pearson test showed a statistically significant indirect correlation ($P = 0.04$) between the mean platelets and plasma ceruloplasmin. During neonatal period of calves, the presence of reticulocytes was observed from the age of one day to 16-18 days. Leukograms revealed that leukocytes were in physiological limits but in the first day of life, their number was above the upper physiological

limit. Calf age had a statistically significant effect on total protein and globulins, Pearson test showed a statistically significant positive correlation ($P = 0.004$) between mean values of ceruloplasmin and total globulins. During the study, it was a progressive increase of haptoglobin in the first days of life (0.15 ± 0.06 g/L) until 3-6 day (0.29 ± 0.07 g/L) followed by a decrease to the 28-29 days (0.14 ± 0.09 g/L). All values of alkaline phosphatase from the examined calves were below inferior physiological limit (24-48 h = 440-512 IU/L, 3 weeks = 247-299 IU/L) indicating the zinc deficiency. Pearson test showed no correlation between changes in alkaline phosphatase values and leukograms in calves. Mean plasma ceruloplasmin in all age groups were below the inferior physiological limit, which indicates a copper deficiency. Iron values increased from calves aged one day to 29 days, the values being within physiological limits. There were no correlations between changes in serum ceruloplasmin and serum iron. Copper deficiency in the 40 calves studied, seems not to be sufficient to cause a microcytic and hypochromic anemia. The results of this study showed that physiological levels of haematological and biochemical profiles of adult cattle may cause diagnostic errors if applied to calves in the neonatal period.

In chapter 9 were made researches regarding the copper, zinc, selenium, iron status and haematological profile according administration of mineral supplement and their correlations between Montbeliarde cattle and their calves. Observations were made in a farm C in France, on a number of 20 Montbeliard cattle divided into two groups of 10 (LCuZn and HCuZn), aged 4-6 years in advanced pregnancy and clinically healthy. Cattle of the HCuZn group received a multi mineral supplement for one month antepartum and one week postpartum. In blood samples taken from cattle and their calves at birth and at 8 days after calving, were made the hematological profile, protein and mineral (Cu, Zn, Fe, Se). Following the clinical examination we found: the average body condition score was 3, undigested faecal fraction score was between 2-3 and faeces consistency scores of 3, perfect scores for dairy cattle. The contents of Cu and Zn in cattle feed was below optimum requirements, so the cattle were deficiented in Cu and Zn. Leukograms showed physiological aspects within the physiological limits, with mention of a larger number of lymphocytes in calves from HCuZn cattle group. At 8 days postpartum, the calves of HCuZn group, had a greater increase in haematological parameters than those whose mothers were not vitamin-mineral supplement. The same situation was observed in cattle from HCuZn group compared with those of LCuZn group, but we have not found statistically significant correlation with hematological profile of their calves. Ration supplementation with zinc, copper and selenium in cattle of HCuZn group, allowed an increase in plasma levels of these minerals until the physiological limits; the calves from HCuZn group showed higher values of copper, zinc, iron and selenium plasma concentrations compared with those in group LCuZn. Copper deficiency in cattle and their calves of group LCuZn, seems not sufficient to cause a microcytic

and hypochromic anemia. Calves in group HCuZn had zinc plasma values that varied in physiological limits.

In chapter 10 were conducted researches regarding the copper, zinc selenium iron status and hematological profile according administration of mineral supplement and their correlations between Charolaise cattle and their calves. Observations were made in a the farm D in France on a number of 20 cattle Charolaise divided into 2 groups of 10 (LCuZn and HCuZn), aged 4-6 years in advanced pregnancy and clinically healthy. For one month antepartum and one month postpartum the cattle from HCuZn group received weekly 50 g mineral supplement. In blood samples taken from cattle and their calves at birth, 8 and 30 days after calving, were performed morphological and biochemical determinations (haematological, protein and mineral profile). Analysis of results enabled the assessment of a body condition score after calving of 3.5, an average of 2-3 in undigested faecal fraction score, a 3 of consistency of feces score, and 3-3,5 refilling of rumen score. Values of hematological profile in cattle and their calves were within the physiological limits. Blood lowest values were observed in calves from cattle of LCuZn group compared with calves from HCuZn group. Pearson statistical test showed no significant correlation between Charolaise cattle and their calves. A few hours postpartum, VEM and HEM have lower values ($P < 0.05$) in cattle and their calves of LCuZn group versus HCuZn group, so that Pearson's test showed statistically significant positive correlation between Charolaise cattle and their calves. Leukograms appearance: lymphocytes, monocytes and eosinophils were more numerous in cattle without vitamin-mineral supplement. In calves, the total leukocytes was higher in those from cattle with vitamin-mineral supplement. Calves of this category had lower levels of neutrophils, monocytes and large lymphocytes. Mean plasma proteins in cattle were located at the lower physiological limit. Few hours postpartum, in calves, was found a hypoproteinemia which suggests a low intake quantitatively and/or quality of colostrum. After birth, the value of copper plasma from both groups of cattle and their calves, were below the physiological limit, but the values were higher in cattle with mineral supplements. At 8 and 30 days postpartum, the amount of copper plasma remained below physiological limits, the higher values were found in calves from cattle with vitamin-mineral supplement, changes being statistically significant ($P = 0.05$). Copper deficiency in cattle and their calves of LCuZn group, seems not sufficient to cause a microcytic and hypochromic anemia. Plasma zinc values in both cattle groups were below the physiological limits, but higher in cattle with mineral supplement, the differences being statistically insignificant. Plasma zinc had higher values in calves from mothers with vitamin-mineral supplement. At 8 and 30 days after calving, zinc values were increased in cattle and their calves of HCuZn group, changes were statistically significant ($P = 0.02$) only in calves. Plasma Fe values in cattle immediately after calving, were below the lower

physiological limit; the higher values of iron in cattle which received mineral supplement. Determination of selenium in cattle and their calves allowed assessment of their selenium deficiencies; the higher values of selenium were in calves from cattle that received vitamin-mineral supplement. Ration supplementation with zinc, copper and selenium in HCuZn group, allowed an increase in plasma levels of these minerals to until to physiological limits, except the selenium plasma, which has remained well below the lower limit of normal. The calves of HCuZn group recorded the higher concentrations of copper, zinc, iron and selenium plasma compared with those in LCuZn group.

The various studies conducted during this work opens up many opportunities, in practice and fundamental. It is appropriate to review the concentrations and evolution of other antagonists of copper and zinc minerals for (Romanian Black Pie, Holstein, Brown Swiss, Montbeliarde and Charolaise) cattle and calves. Analysis of efficacy of copper and zinc supplement in organic and/or mineral form can be made by comparing the blood status and health performance, production and reproduction according to the form of minerals consumed by animals. The cost of supplementation of the ration with vitamin and minerals can be recovered quickly than if the disease prevalence increased or mortality occurrence. Physiological parameters of oligo-elements should be reconsidered by breed and performance of animals studied but also according to the form of trace elements ingested by these animals.