PhD THESIS

Study on the haematological and biochemical profile of *Equus asinus* and evaluation of milk quality parameters

(SUMMARY OF THE DOCTORATE)

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Introduction

Donkey milk has a composition very close to human milk, so it can replace breast milk when appropriate. From the data presented in the literature it can be observed that donkey milk is recommended in the diet of people suffering from food allergies, in people with low immunity and in people suffering from various diseases.

Knowledge of the quantitative and qualitative parameters of milk and colostrum, as well as the haematological and biochemical profile of the donkey are essential to achieve different correlations between these parameters and the health of these animals. Knowledge of the biochemical and haematological parameters of the blood, as well as the influence of the species, breed, age, lactation, exploitation system and physiological state, is also a fundamental tool for the clinical evaluation of animals.

It is important to establish the metabolic profile of the donkeys and to establish the limits for the main biochemical and haematological parameters according to the factors taken in the study. So, certain parameters if they go beyond the permissible limits lead us to the determination of the various disturbances that may be due to poor health. These results are also useful for asin breeders to address these problems. These aspects, if present and not remedied, may lead to damage to the health of the animals, the quantitative and qualitative production of milk and the breeding in the establishment, as well as the productivity and general management of the establishment.

The metabolic profile of milk donkeys is used for nutritional assessment, disease diagnosis and fertility assessment (INGRAHAM et al., 1988). The success of highlighting the metabolic profile very well is limited because it is influenced by diet, the effect of herd, lactation stage, milk production and season, all these factors can affect the concentration of metabolites in the blood (LEE et al., 1978).

Knowing the chemical composition of donkey milk is very important, because milk is of great economic interest to processors. Farmers and processors in this sector of activity, having information on the chemical composition and benefits of donkey's milk, have the opportunity to harness this milk at its true value. Donkey milk can be used in the pharmaceutical industry to obtain various products intended mainly for people suffering from allergies but also as a substitute for breast milk.

The chemical composition of donkey milk is affected by certain factors, such as: lactation stage, number of lactations, age of donkeys, nutrition, climatic conditions, milking technology, geographical area, exploitation technology, animal health and welfare, as in the case of other animal species (DOREAU et al., 1989; GIOSUÉ et al., 2008).

Purpose and objectives of the research

The motivation for this research is to know the hematological and biochemical profile of blood according to the physiological and seasonal status of a population of *Equus asinus* as well as to evaluate certain quantitative parameters of milk. The topic addressed in this doctoral thesis is a novelty due to the fact that in our country and internationally there is limited information for donkey milk and for the metabolic profile of donkey (*Equus asinus).*

The purpose of the thesis. Assessment of the haematological and biochemical profile of the blood according to the physiological and seasonal status of Equus asinus; the study of the quality parameters of milk, i.e. the composition of milk and colostrum; determination of the antioxidant capacity of milk according to lactation and heat treatments as well as evaluation of some milk contaminants.

Objectives of the work

Objective 1. Evaluation of the biochemical and haematological profile of the blood in the donkey under the influence of lactation and under the influence of the collastral period. Another aspect pursued for the biochemical and haematological profile was the determination of the influence of the season on these parameters.

Objective 2. Milk quality assessment: physico-chemical and microbiological parameters of milk and physico-chemical parameters for colostrum at *Equus asinus*.

Objective 3. Determination of the antioxidant capacity of donkey milk under the influence of lactation as well as determination of changes for antioxidant capacity in colostrum according to the postpartum day. Comparison of data obtained for antioxidant capacity in milk and donkey colostrum in the Transylvanian area with data from literature. Changes in the thermal treatments (boiling, freezing and freeze-freezing) on total antioxidant capacity were also evaluated for donkey milk.

Objective 4. Assessment of the level of heavy metals and some mineral elements in milk according to lactation (lactation I-IV). Determination of heavy metals and mineral elements in donkey's milk and comparison with other results from the literature and legislation in the field for these types of residues.

Biological material. The biological material studied is represented by Equus asinus. A total of 50 donkey heads from the Transylvanian area (Salaj, Hunedoara and Cluj) were studied.

Structure of doctoral thesis

The doctoral thesis entitled "Study on the haematological and biochemical profile of Equus asinus" and evaluation of milk quality parameters, is structured in two parts, namely: the first part refers to the current state of knowledge and the development of a number of 3 chapters and part -2 presents its own research comprising 6 chapters, conclusions, originality and innovative contributions of the thesis and bibliography. The thesis is spread over 160 pages and comprises 50 tables and 29 figures and 317 bibliographic references.

The first part of the doctoral thesis comprises the "Current State of Knowledge" and comprises a number of 29 pages. **Chapter 1** outlines the aspects of the phylothenia and taxonomy of the Asins, the breeds of asin as well as the growth of the asin in our country and worldwide. **Chapter 2** of the first part presents physiological status at Equus asinus and haematological and biochemical parameters under the influence of lactation and the postpartum period at Equus asinus. **Chapter 3** presents general aspects of donkey's milk. The chemical composition is presented under the influence of various factors, such as: lactation, season, nutrition. Factors influencing the quality of donkey's milk (genetic, physiological, technological) are also highlighted.

Part 2 of the doctoral thesis comprises a number of 116 pages. **Chapter 4** shows: the purpose, objectives of the study, experimental design and experimental activities carried out during the research.

Chapter 5 shows the biological material studied and the working methods. Presentation of the methods used for the analysis of the biochemical and haematological parameters of the blood at the donkey and the methods for the physico-chemical analysis, NTG, NCS, antioxidant capacity and residues of heavy metals in milk.

Chapter 6 presents the physiological status of Equus asinus and the hematological and biochemical profile in donkey under the influence of lactation and season. In the postpartum period, haematological and biochemical parameters were analyzed according to the postpartum day and day 30 lactation.

Chapter 7 tells the quality of donkey's milk. The physico-chemical composition and NTG and NCS for donkey milk were analysed according to lactation and under the influence of feed. Another aspect studied was the caracerization of the physico-chemical composition of the colostrum under the influence of the day postpartum.

Chapter 8 is assigned to the study on the antioxidant capacity of milk and donkey colostrum. The antioxidant capacity of milk under the influence of lactation, the area of provenance of the samples, as well as the influence of heat treatments on the physico-chemical composition and antioxidant capacity of milk was analyzed. Antioxidant and donkey colostrum capacity was analyzed under the influence of the postpartum day.

Chapter 9 contains the results on residues of heavy metals and mineral elements in milk according to lactation. **Chapter 10** presents the general conclusions

and recommendations and **Chapter 11** presents the originality and innovative contributions of the thesis.

Research results

Chapter 6 presents the results on physiological status at Equus asinus under the influence of lactation and colostrum day. Evaluation and monitoring of physiological parameters is essential in order to be able to assess the health of animals according to lactation. WBC (G/l) in lactation 1 has an average value of (9.66±0.56) (G/l) and (13.57±0.80) (G/l) in lactation 4. These values are in line with those published by (HORÁČKOVÁ et al., 2017) on haematological parameters in the donkey. Hb has an average value of (114.08±2.89) (g/l) in lactation 1 and Hb increases reaching the highest values in lactation 4, (131.30±3.56) (g/l). These values are within the limits allowed for this parameter. The haematological values obtained in this study for lactation-based donkeys are in line with those reported in other studies (CALDIN et al., 2005; BURDEN et al., 2016; LAUS et al., 2015).

Metabolic profile and blood parameters show significant variations when it comes to animals of different sex and using different management systems, or housed differently. Certain parameters can be used as indicators of changes that take place and under the influence of housing and management conditions. These indicators are (MVC, RDW) (SILVA et al., 2018). The decrease in the number of erythrocytes causes anemia. The haematological parameters analyzed vary significantly under the influence of lactation at the donkey and fall within the specific ranges for asins (LONGODOR et al., 2019). It can be seen from the values obtained in the case of the donkeys studied that the values fall within the specific limits for asins, with changes depending on the factors that were studied (lactation and season). NEU (%) changes their values under the influence of lactation as follows: in lactation I, it has an average value of (34.54±1.60) (%) and (51.08±2.23) (%) lactation IV. LYM (%) ranges from (38.72±0.69) in lactation I and (52.98±2.96) in lactation 2. Results obtained for NEU (%) and for LYM (%) significantly influenced by the number of lactations. Aspect reported by other authors such as (SEDLINSKÁ et al., 2017; COROIAN, 2013; BARKER, 1998, 2004; GLUCKMAN and HANSON, 2005, 2006).

In the case of haematological parameters, significant changes can be observed under the influence of lactation as follows: WBC (G/l) has the lowest mean value in lactation I, (9.66±0.56) and increases in the following lactations, reaching the highest mean value in lactation IV, (13.57±0.80). Hb (g/l) behaves similarto WBC, reaching the highest average values in lactation IV (131.30±3.56). LYM (%) have the lowest values in lactation I, (38.72±0.69) and lactation 2, 3 and 4 have similar mean values. MON (%) thus manifests itself, under the influence of lactation, having an interval between (1.14±0.16), lactation I and (1.42±0.17) in lactation IV. It can be said that lactation influences the mean values for haematological parameters in the donkey. Most reaching the lowest average values in the first two lactations and the highest in lactations 3 and

4. The haematological parameters studied under the influence of lactation fall within the specific limits published by other authors for this species and taking into account lactation or other influence factors. Hematological parameters such as WBC, Hb, HCT, MCV, NEU, LYM, MON, NEU, LYM, EOS, PLT, MCHC and BAS were significantly affected by the number of lactations.

There are several factors that can influence the biochemical parameters of the blood and the hematological parameters even within the same species, namely: nutritional factors, aspects of the area of provenance, mode of exploitation, sex, age, lactation (HORÁČKOVÁ et al., 2017; MUSHI et al., 1999; SEDLINSKA et al., 2016; SOW et al., 2012; Mori et al., 2004). The total protein (g/l) varied in the range (61.28±3.36) (g/l) in lactation I and (67.86±1.96) (g/l) in lactation IV. Glucose (mmol/l) behaves similar to to the total protein, showing the lowest values in the first three lactations and the highest values in lactation IV.

Triglycerides (mmol/L) under the influence of lactation fall within the range (0.66±0.08) (mmol/L), lactation I and (0.85±0.09) (mmol/L), in lactation IV. Total calcium (mmol/L) has the highest values in lactation I, II and IV and the lowest in lactation III. Magnesium (mmol/L) changes its values according to lactation.

Parameters, glucose, creatinine, potassium, ALT, AST, ALP, GGT and CK were significantly affected by the number of lactations. For all these parameters, the highest values were observed in lactations 2, 3 and 4, while the lowest values were observed for L1. For urea, total protein, albumin, cholesterol, triglycerides, total calcium, sodium, total bilirubin and for magnesium no significant difference between lactations was observed. The haematological and biochemical profile of the donkey was also evaluated according to the postpartum day and on day 30 of lactation.

The colotral period produces significant changes in the hematological and biochemical profile of the donkey. These aspects are raised in this objective of the thesis. The haematological and biochemical parameters of the blood were analyzed under the influence of the postpartum day (day 1 and day 5) and on day 30 of lactation.

Most biochemical parameters of the blood are significantly influenced by the postpartum period. Biochemical parameters show a high mean value on the first day postpartum (highest values) and these decrease on day 5 postpartum. The lowest values of these biochemical parameters were recorded on day 30 of lactation, while the highest on day 0-1 postpartum. By contrast, for CK the highest values were obtained on day 30 of lactation (246.80 U/L), followed by day 0-1 postpartum (218.40 U/L) and day 0-5 postpartum (206.60 U/L).

Chapter 7 shows the quality of milk in the donkey. The fat was significantly higher in L3 and L4 than L1, but no statistical difference between L3, L4 and L2. Lactose in milk was significantly higher in L2 compared to other lactations. The water content was higher in L4 compared to L1 and no significant difference was observed L2 and L3. As regards protein, no significant difference between lactations was found. Lactose showed the lowest average values in lactation 1, (6.67±0.06) and the highest in lactation

2, (7.14±0.01). The water content varied in the range (83.83±0.82) in lactation 1 and (88.21±0.64) in lactation 4.

Average lactose values and water content fall within the values that are reported in the literature (BILANDZIC et al., 2014; PILLA R., 2010; MURUA et al., 2013). The variability of milk production reported in the literature is influenced by a significant number of factors such as: nutrition, individuality, genetics, reproduction and management of growth and exploitation (SALIMEI, 2012). NCS recorded the highest value in L4 (357.44), while the lowest in L1 (292.40). In the case of NTG, the highest values were obtained in L4 (90.64) and L3 (84.48), followed by L2 (64.12) and L1 (52.12).

In **subchapter 7.1**, the physico-chemical composition of the colostrum was also evaluated in the donkey. The fat, protein, lactose and water content was affected by the postpartum day. The highest values for fat and lactose were obtained on day 5, while the lowest on day 1. The protein content, the lowest significant value was obtained on day 1 compared to the others. While the higher water content in the colostrum was found on days 4 and 5.

Chapter 8 presents the assessment of the antioxidant capacity of milk and donkey colostrum. Antioxidant capacity in milk and dairy products is influenced by many factors such as: species, lactation, lactation period, breed, age, nutrition and thermal processes applied (boiling, pasteurization, sterilization, freezing, freeze-drying). The total antioxidant capacity in donkey milk varies between 16.02 (U/ml) and increases in lactations II and III, reaching the highest values in lactation IV, 17.55 (U/ml), in the case of donkeys in the Cluj area. The donkeys in the Salaj area show variations in the 15.68 (U/ml) in lactation I and 17.63 (U/ml) in lactation IV. These values are similar to those reported by (LING et al., 2018) on donkey's milk. Donkey milk has changes under the influence of applied processes for fat, protein and lactose. Donkey milk is a food beneficial to the human body, due to its physico-chemical composition and antioxidant capacity. Antioxidant capacity is high, which is recommended in the diet of sensitive people and suffering from various diseases.

In the study conducted on the donkey colostrum over a number of 5 days, it can be observed that the highest values for TAC (U/ml) are in the first days postpartum, and decrease towards the end of the colostrul period. We observe in the case of samples collected and analyzed on day 30 of lactation, the lowest values. The range of TAC (U/ml) is presented as follows: day 1 postpartum 35.06 (U/ml) and day 5 postpartum 26.91 (U/ml) and on day 30 lactation decreases to 21.48 (U/ml).

Chapter 9 shows the mineral elements and some heavy metals in the donkey's milk. Following analyses of heavy metals from milk harvested from Cluj and Sălaj and nearby towns, the level of heavy metals is high. Thus it can be confirmed that, due to the intense pollution in these areas, raw milk, presents residues of heavy metals. Higher values for Cu and Al were recorded in L4, while the lowest values in L1. Milk had significantly higher levels of Fe and Zn in L1 compared to L2, L3 and L4.   Na   levels were significantly lower in L1 compared to other lactations and no significant difference

between L4 and L3 was observed. In the case of Mg, the lowest level was observed in L1 and L3, while the highest in L4. The Al level was higher in L4 than L2 and L1 and no significant difference between L3 and L4 was observed. With had the highest level in L4 compared to other lactations and no significant difference was found between L1, L2, L3 and L4.

General conclusions

Conclusions on (objective 1)

1. The establishment of the haematological and biochemical profile at the donkey is a basic test that is carried out in order to establish the values for these parameters. The health status of animals may be assessed on the basis of these indicators. Regarding the influence of lactation on the haematological profile it can be said that these parameters are influenced by lactation and the mean values increase from lactation I to lactation IV.

2. Hematological and biochemical parameters according to lactation fall within the ranges encountered in the literature. There were no very high values for these parameters that can produce metabolic imbalances. It is worth noting that advanced lactation determines the highest values for all biochemical and haematological parameters in the donkey.

3. In large farms, haematological and biochemical tests may be routine tests aimed at early diagnosis of diseases and the possibility of eliminating treatment with medicinal products, if these metabolic disorders are detected very quickly.

4. The colastral period produces significant changes for both biochemical and haematological parameters. It has been observed that in the postpartum period, the postpartum day alters the hematological and biochemical parameters. These parameters were compared with the parameter values of day 30 lactation.

Conclusions on (objective 2)

1. The physico-chemical composition (fat, protein, lactose and water content) of donkey milk (Equus asinus) is influenced by lactation. Fat has the lowest average values in lactation 1. These values increase in the next three lactations and reach the highest average values in lactation 4. Protein is another very important physico-chemical indicator for donkey milk and has the highest content in lactation 1 and 2 and the lowest content is in lactation 3.

2. The microbiological load (NTG and NCS) of donkey milk is influenced by lactation, an increase can be observed for both NTG and NCS as the number of lactations increases. Both the NTG and the NCS fall within the limits set by the legislation in force

which refers to these quality parameters. A microbiological load is observed which corresponds to the rules in force even if the milking is done manually. Appropriate hygiene for milking and the health of the donkeys determines a quality milk which corresponds from a compositional point of view.

3. The donkey colostrum under the influence of the postpartum day shows significant changes for physico-chemical parameters (fat, protein, lactose and water). The physico-chemical parameters analyzed showed an increase from day 1 postpartum to day 4 postpartum. On day 4 postpartum all parameters analyzed reach the highest average values.

Conclusions on (objective 3)

1. It is very important that the thermal process applied has the smallest changes. Donkey milk has a total antioxidant capacity that increases with the number of lactations. This has been observed for all animals studied, regardless of the area of provenance. The highest level on TAC is lactation 3 and 4.

2. Changes to TAC in donkey milk have occurred by applying the boiling, freezing and freeze-dried process. In donkey's milk, the lowest values are in the case of freeze-dried milk, followed by boiled milk and the least affected is frozen milk. Frozen milk has undergone the smallest changes in this parameter compared to raw donkey milk.

3. The TAC in the donkey colostrum changes its contents under the influence of the postpartum day. TAC in the first days postpartum shows high values compared to the last days of the colastral period, values that are in line with those highlighted in milk on day 30 of lactation.

Conclusions on (objective 4)

1. The residues of metals in donkey's milk were analysed on four lactations (lactation 1-4). We considered it appropriate to establish these contaminants in donkey milk and due to the fact that this milk can be used for sensitive categories of persons (people suffering from allergies and with various diseases).

2. The average content for each metal analysed increased by the number of lactations. These contaminants exhibited similar behaviour to other microbiological contaminants (NTG, NCS). It can be said that the possibility of retention of contaminants increases as the number of lactations increases.

Recommendations

Donkey milk has a chemical composition similar to breast milk. The high lysozyme content is recommended for people suffering from food allergies or various respiratory conditions. The heat treatments that have been used on donkey milk have led to smaller changes for boiled and frozen milk. It is recommended to use the freezing process for donkey milk as it produces the smallest changes in chemical composition and antioxidant capacity and can thus be preserved for a longer period.

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