

Article

Analysis of Environmental Factors' Impact on Donkeys' Colostrum Quality

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Abstract: Colostrum is a natural product, issued by both mammals and humans in the first week of lactation. Among different species, donkey colostrum is considered to have, in addition to a valuable composition in nutrients and immune factors, an outstanding similitude with human colostrum. In this context, and taking into account the scarcity of available data concerning the interaction between climate factors and colostrum quality, a trial was conducted aiming to identify the possible influence of environmental factors on donkey colostrum nutritional traits. A stock of 175 jennies from 7 farms located in Cluj and Sălaj Counties was analyzed over a 7-day postpartum period. During the experimental period, the daily temperature, humidity, and wind velocity data were collected. Strong positive correlations are reported between the studied colostrum nutritional traits (fat, protein and lactose). Testing the impact of some environmental parameters upon nutritional content of donkey colostrum indicated three factors that have an influence on the nutritional quality of donkey milk colostrum, labelled as: colostrum nutritional traits, environmental air traits and some colostrum nutritional traits, and climatic traits and some colostrum nutritional traits.

Keywords: coefficient of variation; correlation; factor; jenny; principal components analysis

1. Introduction

In recent decades, a strong interest in the milk delivered by animals other than cows has been observed [1,2]. In the particular case of donkeys' milk, one may find that it has recently gained more interest from researchers because it is highly tolerated by infants with protein allergies [3]. Other studies [4,5] emphasize the particularity of donkey milk because of its rich lysozyme content, making it suitable for probiotic beverages. Cosentino et al. [6] highlighted that in the context of the increasing importance of diversity preservation, even in the most vulnerable areas of Natura 2000, because of the multiple uses of donkey milk (even for newborns), donkey rearing should be encouraged.

According to Oxford Dictionary of Food and Nutrition [7], colostrum is "the milk produced by mammals during the first few days after parturition; compared with mature milk, human colostrum contains more protein (2 compared with 1.3 g/100 mL), slightly less lactose (6.6 compared with 7.2 g/100 mL), considerably less fat (2.6 compared with 4.1 g/100 mL), and overall slightly less energy (56 kcal (235 kJ)/100 mL compared with 69 kcal (290 kJ). Colostrum is valuable source of antibodies

for the newborn infant. Animal colostrum is sometimes known as beestings and human colostrum as foremilk”.

The benefits of both human and mammal colostrum result as a consequence of its composition. In addition to valuable nutritional components, the composition of colostrum includes growth (growth factors such as epidermal, insulin-like, etc., and growth hormone), immune (lactoferrin and immunoglobulins), and anti-inflammatory factors, with roles in reparation of damaged cells, regulation of the immune response, or in reducing inflammatory disorders [8–10]. Within the first days after birth, both human and mammal newborns face a potentially harmful environment, due to microbial threat [11]. In this context, the outstanding colostrum capacity for postnatal transport of immunoglobulins from mother to newborn can be mentioned [12].

Colostrum quality is of primary importance in human nutrition. For this reason, the preoccupation in the field, concerning the replacement of the human colostrum with other species colostrum, is reflected by literature [8–10,13,14]. This is the appropriate context in which to mention the opportunity of using colostrum from donkeys as a human colostrum replacement. The fundamentals of this assertion are based on the already-known fact that donkey milk, as result of its composition, may be successfully used as a replacement for human milk [15–22]. The reason donkey milk is so similar in macro-composition to that of human milk is still unclear, especially given that donkeys and humans are not phylogenetically related [23].

According to FAOSTAT [24] statistical data, the global development of donkey rearing over the last 25 years, has exhibited a variety of trends. Africa, Asia and the Americas are the continents in which donkey rearing is best represented, while Europe and Oceania have smaller stocks. While Asia possessed the most significant stock in terms of the number of donkeys reared at the beginning of the studied period, in the end of the analyzed period in 2014, Africa was placed in first place. One may also note that, except for Africa, where a positive trend is emphasized, on the other continents, donkey rearing exhibits a decreasing tendency from 1990 to 2014. In the European Union (EU), the mean number of donkey stocks for the analyzed 25-year period comprises 611,785 individuals, with the minimum number of 411,135 individuals being reported in 2014, and the maximum of 928,274 individuals, in 1990 (FAOSTAT Data). The study of the evolution of donkey rearing in Romania indicates that it represents 5% of total EU donkey stocks. A donkey stock of 31,000 is the mean for the 25-year period in Romania, with a minimum of 29,000 reared in 2005, and a maximum of 35,000 reared in 1990 [24].

Environmental factors, mainly heat stress, affect the behavior and wellbeing of mammals, generally speaking, and donkeys in particular [25,26], the quality of donkey milk [27–32], and also the production and quality of colostrum, generally speaking [33,34]. Studies upon pigs show that valuable components of colostrum, as immunoglobulins, are reported in higher amounts when low temperatures are recorded [35], corresponding to the farrowing of that place in winter, compared to values reported in colostrum when farrowing takes place in spring, summer, or even fall [36]. Even though, in recent years, research has been conducted in Romania emphasizing donkey milk quality and composition [37–39], no data concerning the influence of environmental factors (such as noise, temperature, humidity, wind, etc.) on colostrum quality and composition are available. It may be useful for the Romanian animal breeding sector, to develop the few research projects that have been conducted to date, taking into account that it is a country with real opportunities for developing the donkey breeding sector for dairy production, especially due to the valuable properties of donkey milk colostrum, and the possibility of supplying added value to nutritional solutions for infants. In this context, this trial aims for a completely new approach in quantifying the donkey milk colostrum nutritional traits in terms of fat, protein, and lactose, by studying this issue in an environmental context, meaning to assess the influence of temperature, relative humidity, and wind velocity on the above-mentioned nutritional traits of donkey colostrum, within regionally specific environmental conditions.

Literature Review

Over a long time period [40,41], researchers have been preoccupied by the roles played by donkeys not only at a farm level, but, in recent decades, also concerning donkey production in terms of meat, skin, and milk. Among these productions, one may notice that a particular interest has been focused on donkey milk, due to the special properties it possesses.

In this respect, we have to mention that colostrum is considered the most valuable component of donkey milk, even though little research has been conducted in this field. Several studies conducted on donkey milk in terms of composition emphasized the lipid content, characterized by a high occurrence of essential fatty acids, with special importance for the diet of infants with Cow Milk Protein Allergy [42], and also the content of essential elements, which emphasize balanced contents of both macro-elements K, P, Ca, Mg, Na, and micro-elements, Cu, Zn, Ni, Cd, Fe [17,43,44].

The most important properties of donkey milk, which include antiviral, antibacterial, and anti-inflammatory properties [45–47], resulting in low pathogen occurrence, may be the consequence of the health status of the donkey's mammary gland, which could be related to the innate immunity of the jennies' udders [48]. Research performed by Yvon et al. (2018) in mice indicate that consumption of donkey milk has anti-inflammatory effect, leading to a reduction of microbiota imbalance in the studied mice [49]. Also, research conducted by Mao et al. (2009) indicated the potential anti-tumor and anti-proliferative role of donkey milk active components [50].

Lactoferrin, b-lactoglobulin and lysozyme are considered the main representatives of the antimicrobial proteins identified in donkey milk and donkey milk colostrum, mainly represented by the role played by antimicrobial agents. During the lactation period, these proteins suffer significant changes in their profile. Therefore, it is considered that colostrum has higher lactoferrin and b-lactoglobulin concentrations compared to raw milk, while the lysozyme concentration was detected at similar levels in colostrum and fresh raw milk [51].

In the last decade, because of anti-allergenic properties, mainly in infants exhibiting Cow Milk Protein Allergy [52–54], donkey milk has received interest as a breast milk substitute, mainly due to its colostrum content. Thus, the study of the β -lactoglobulin, identified in two molecular forms— β -lactoglobulin I, and β -lactoglobulin II—in donkey milk and colostrum, respectively [55]. In this respect, we mention that molecular studies conducted by Criscione et al. (2018) led to identification of a new allele, named F, which is believed to be associated with a severely reduced expression of β -lactoglobulin II, or null expression of the previously mentioned β -lactoglobulin II [56].

If studies concerning donkey milk and donkey milk colostrum production, uses, traits, and composition are available, the same cannot be said about studies focusing on the influence of environmental factors upon the same issues related to donkey milk and colostrum. Several studies have shown that heat stress has a negative effect on the quality and quantity of donkey milk. Thus, research conducted in different areas of Italy emphasizes higher milk yields in donkeys foaling in the cold season, meaning autumn–winter, compared to milk yields resulting from donkeys foaling in the warm season, meaning the spring–summer period [15,57,58]. According to experiments performed by Ragona et al. (2016) on Amiata donkeys, the warm seasons positively influence the lactose and casein contents in milk, as well as milk yield [20]. Faye and Konuspayeva (2012) show that tendencies have been recorded in the natural creation of ecosystems for certain dairy species, including donkeys [59].

The present study is structured in a manner that is based on updated knowledge concerning donkey milk and donkey milk colostrum as emphasized by the literature review. We describe the methodology of sampling and data analysis, as well as results concerning the complex interrelationships between the analyzed environmental factors and donkey milk colostrum within the context of the specific conditions of the experimental area. The implications of this trial in terms of promoting sustainability at a national level are also approached. Meanwhile, the limitations of this research are also emphasized, together with appropriate directions for further studies in this field.

2. Materials and Methods

2.1. The Experimental Area

The experiment was conducted on seven private donkey farms, located close to two urban areas, and characterized by similar climatic conditions. One urban area is by Huedin town (3 private farms), and the other is Zalău town (4 private farms). Huedin is located in Cluj County, at the coordinates: 46°52'00" N, and 23°02'00" E. Zalău town is located in Sălaj County, at the coordinates: 47°11'28" N, and 23°03'26" E. The Huedin area is characterized by an annual mean maximum temperature of 13.66 °C, a mean minimum temperature of 5.58 °C, and a mean annual precipitation of 682.5 mm (calculated, as an average of the last 30 years [60]). Zalău area is characterized by an annual mean maximum temperature of 13.48 °C, a mean minimum temperature of 5.54 °C, and a mean annual precipitation of 634.3 mm (calculated, as an average by the last 30 years [61]). The trial was carried out on 25 jennies at each farm, during the 7-day postpartum period, during which they fed the offspring with colostrum, from the 2–8 May 2016 in Cluj County, and the 4–10 May 2016 in Sălaj County.

2.2. Sample Collection

The colostrum samples were collected once a day, in the morning, from 175 multiparous jennies during the experimental period. The females received the same feed, and were maintained in stabulation during the first 7 days of milking, when colostrum is released. The colostrum samples, collected daily in an amount of 10 mL, were delivered to the laboratory, and were maintained at a temperature of 4 °C. The environmental temperature, relative humidity, and wind velocity were continuously monitored during all 7 days of the experimental period, using WE900 Weather Station (4–20 mA) monitoring station, produced by Global Water, USA. The most important component of the climate traits in the experimental area is temperature, because an increase of 0.9 °C has been reported for the last 10 years (Romanian National Administration of Meteorology. Climatic monitoring).

2.3. Data Analysis

Qualitative and quantitative colostrum traits were analyzed with Lactoscan MCC device, produced by Milkotronic Ltd. (Nova Zagora, Bulgaria). Fat, protein, and lactose determinations were performed by direct measurement, and the basic principle of the methodology is the real-time measurement of the speed of the ultrasound in the colostrum.

IBM SPSS Statistics v. 20 (IBM, Armonk, New York, USA) was used for raw colostrum and climatic data processing. To emphasize both colostrum and climatic traits, descriptive statistics was used. Furthermore, factorial analysis, based on the Principal Components Analysis (PCA) technique, was used in order to emphasize the main dimensions among the 8 considered variables (environmental factors-temperature, °C; humidity, %; wind velocity, m/s; and colostrum pH and composition-fat, protein, lactose, and water, %). To conduct the PCA, because the variables do not have the same units of measurement, they must previously have been standardized. In this case, the correlation matrix obtained for the standardized data is equivalent to the variance-covariance matrix of the unstandardized data [62,63]. Before applying the SPSS Principal Components Analysis, the fitness of data was determined by applying the Bartlett's test of Sphericity, and Keiser-Meyer-Olkin test for measuring sampling adequacy, with data with values above 0.6 being considered [62]. The Eigenvalue criteria were used in extracting factors. Factors with Eigenvalues equal to or greater than one were considered significant and were retained [63]. All items with factor loadings less than 0.5, and with communality scores of less than 0.2, were eliminated [63]. To estimate the reliability of each item, the Cronbach's alpha reliability coefficient was calculated, and items with values above 0.6 were retained [62,63]. The principal components were identified, taking into account the variables most strongly correlated with each considered component. Correlations above 0.5 were taken into consideration.

3. Results and Discussion

During the 7-day experimental period, the mean temperature was 12.30 °C, while for mean relative humidity and mean wind velocity, values of 64.43%, and 8.43%, respectively, were reported (Table 1). According to the values of the coefficients of variation, the means of temperature (CV = 9.67%), and relative humidity (CV = 12.75%) were representative for the study, while the mean wind velocity (CV = 20.16%) had satisfactory representativeness. The mean fat content identified in donkey colostrum during the research period was 3.77%, while the protein was 2.36%, lactose was 2.35%, water was 86.37%, and pH was 6.96. The fat content exhibited a high variation during the colostrum secretion period, from 1.89% up to 4.88% (CV = 20.21%), with smaller variations being identified in protein content (1.47%–2.75%) and in lactose content (1.43–2.71%), which were characterized by coefficients of variability of CV = 11.95% and CV = 11.86%, respectively (Table 2).

Table 1. Descriptive statistics concerning the evolution of the climatic parameters within the experimental area, 2–8 May 2016.

| Parameter | N | Temperature (°C) | Humidity (%) | Wind Velocity (m/s) |
|--------------------------|---|------------------|--------------|---------------------|
| Mean | 7 | 12.30 | 64.43 | 8.43 |
| Standard deviation | 7 | 1.19 | 8.22 | 1.70 |
| Minimum | 7 | 10.00 | 56.00 | 5.00 |
| Maximum | 7 | 14.00 | 74.00 | 11.00 |
| Coefficient of variation | 7 | 9.67 | 12.75 | 20.16 |

Table 2. Descriptive statistics for the evolution of donkey colostrum traits, 2–8 May, 2016.

| Parameter | N | Fat (g/100 mL) | Protein (g/100 mL) | Lactose (g/100 mL) | Water (%) | pH |
|--------------------------|-----|----------------|--------------------|--------------------|-----------|------|
| Mean | 175 | 3.77 | 2.36 | 2.35 | 86.37 | 6.96 |
| Standard deviation | 175 | 0.76 | 0.28 | 0.26 | 2.12 | 0.15 |
| Minimum | 175 | 1.89 | 1.47 | 1.43 | 82.50 | 6.70 |
| Maximum | 175 | 4.88 | 2.75 | 2.71 | 89.10 | 7.20 |
| Coefficient of variation | 175 | 20.21 | 11.95 | 11.86 | 2.46 | 2.19 |

When comparing the results of our research (Table 2), with the values identified in other mammals and humans (Table 3), it can be seen that donkey colostrum nutrient content has the greatest quantitative similarity with human and cow colostrum. For example, the mean fat content (3.77 g/mL) identified in donkey colostrum is quantitatively closer to the fat content in cow colostrum, as reported by Meyer and Kamphues [64], and human colostrum, as reported by other research [65,66], compared to the fat content of sow colostrum reported by Park to be 5.8% [41], and of ewe and she-goat, as reported by Meyer and Kamphues [64]. The donkey colostrum mean protein content of 2.36 g/100 mL found by our research (Table 2) falls within the interval of values reported for human colostrum protein [65,66], while it has smaller value than the protein content of cow, sow, ewe and she-goat colostrum (Table 3) [64,67].

Table 3. Composition of colostrum in different livestock species and humans.

| Issue | Fat | Protein | Lactose | Unit | References |
|-------|----------|----------|---------|----------|------------|
| Human | 2.9–2.95 | 2.29–3.7 | 5.3–5.7 | g/100 mL | [39,40] |
| Cow | 3.6 | 13 | 3.1 | % | [38] |
| Pig | 5.8 | 10.6 | 3.4 | % | [41] |
| Sheep | 12.4 | 13 | 3.4 | % | [38] |
| Goat | 9 | 8 | 2.5 | % | [38] |

According to our research, the mean lactose content in donkey colostrum (2.36 g/mL) is quantitatively close to the lactose content from she-goat colostrum (Tables 2 and 3), reported by Meyer and Kamphues [38], but is lower than the lactose content of sow, cow, and ewe [64,67,68], and much lower (Table 3) than the lactose content in human colostrum [65,66]. In our research, the evolution of colostrum nutrients-fat, protein, and lactose-between the first and the 7th day of colostrum delivery exhibits an increasing trend, even if individual fluctuations can be observed.

Strong correlations are identified between the donkey colostrum mean fat content on one hand, and mean protein and lactose content, on the other hand ($R = 0.653$ and $R = 0.799$, respectively). Also, a strong positive correlation, $R = 0.638$, was found between colostrum protein and lactose (Table 4).

With regard to the interactions between donkey milk colostrum nutrients (fat, protein, lactose, etc.) and environmental factors, no studies were found. If many references concerning the effects of heat stress on the nutritional composition of dairy species milk, are available [27,28], when referring to donkey colostrum, a notable scarcity of data may be noticed.

The nutritional characteristics of donkey colostrum analyzed in this study were very weakly, moderately and strongly correlated with climatic factors. Moderate and strong correlations are emphasized using bold numbers in Table 4. The temperature is negatively moderately correlated ($R = -0.510$) with protein from donkey colostrum, while the correlations between air temperature and the other colostrum nutrients analyzed in this study were also negative, but weak. Strong positive correlations were reported between air relative humidity and colostrum fat and protein content ($R = 0.644$ and $R = 0.655$, respectively), and a positive moderate correlation was found between air relative humidity and colostrum lactose. Weak negative correlations were observed between wind velocity and colostrum nutrients, as analyzed in this study (Table 4).

Table 4. The correlation matrix of the donkey colostrum traits and environmental items considered within this experiment, May 2016.

| Issue | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|-------|-------|--------------|--------------|--------|-------|---------------|--------------|---------------|
| 1 | 1.000 | 0.653 | 0.799 | −0.274 | 0.202 | −0.219 | 0.644 | −0.235 |
| 2 | | 1.000 | 0.638 | −0.209 | 0.017 | −0.510 | 0.655 | −0.207 |
| 3 | | | 1.000 | −0.331 | 0.142 | −0.140 | 0.518 | −0.149 |
| 4 | | | | 1.000 | 0.043 | −0.090 | 0.009 | 0.033 |
| 5 | | | | | 1.000 | 0.126 | −0.163 | 0.140 |
| 6 | | | | | | 1.000 | −0.619 | 0.095 |
| 7 | | | | | | | 1.000 | −0.506 |
| 8 | | | | | | | | 1.000 |

1-Fat (g/100 mL), 2-Protein (g/100 mL), 3-Lactose (g/100 mL), 4-Water, %, 5-pH, 6-Temperature (°C), 7-Relative humidity (%), 8-Wind velocity (m/s).

After data standardization, according to the Eigenvalues (equal to or greater than one), three factors were considered significant and were retained. For the same three factors, the Cronbach's alpha reliability coefficients show values above 0.6, with an overall reliability of 0.71. Based on the Chi-square value of 4430.03 ($p < 0.001$), the results of the Bartlett's test of Sphericity for our data emphasize the significance and the Keiser-Meyer-Olkin test result of 0.63 confirmed the sampling adequacy.

The 3 principal components identified, taking into account the variables most strongly correlated with each considered component (Table 4), are presented in Table 5. The first component, labeled as "colostrum nutritional traits", has a reliability coefficient of 0.74, and explains 39.12% of the total variance, which may be explained by the importance of colostrum nutritional value, regardless of environmental conditions. This factor is composed of three nutritional components-fat, protein, and lactose—having a mean of 2.82 with $SD = 0.321$. The second factor, which is labeled as "environmental air traits and some colostrum nutritional traits" is responsible for 17.99% of variance, and has a reliability coefficient of 0.69, with a mean of 20.89, and $SD = 0.915$ (Table 5). It is less important than the

first factor, but it suggests the influence of environmental stress (air temperature and relative humidity) on fat and lactose content in donkey milk colostrum.

Table 5. Principal component analysis on colostrum traits, and environmental variables.

| Eigenvalue | Variance % | Factor | Item | Factor Loading | Communalities | Mean | SD |
|------------|------------|---|-----------------------|----------------|---------------|-------|-------|
| 3.12 | 39.12 | Colostrum nutritional traits $\alpha = 0.74$ Mean = 2.82 SD = 0.321 | Fat | 0.916 | 0.840 | 3.77 | 0.762 |
| | | | Protein | 0.818 | 0.662 | 2.36 | 0.281 |
| | | | Lactose | 0.801 | 0.659 | 2.35 | 0.280 |
| 1.43 | 17.99 | Environmental air traits and some colostrum nutritional traits $\alpha = 0.69$ Mean = 20.89 SD = 0.915 | Air temperature | 0.751 | 0.730 | 12.85 | 1.556 |
| | | | Air relative humidity | 0.722 | 0.561 | 64.62 | 6.231 |
| | | | Fat | 0.689 | 0.496 | 3.77 | 0.281 |
| | | | Lactose | 0.614 | 0.452 | 2.35 | 0.280 |
| 1.07 | 13.45 | Climatic traits and some colostrum nutritional traits $\alpha = 0.62$ Mean = 4.71 SD = 0.432 | Wind velocity | 0.722 | 0.618 | 8.02 | 2.064 |
| | | | Fat | 0.602 | 0.551 | 3.77 | 0.762 |
| | | | Protein | 0.582 | 0.433 | 2.36 | 0.281 |

The third factor, labeled as “climatic traits and some colostrum nutritional traits” is responsible for 13.45% of variance, and has a reliability coefficient of 0.62, with a mean of 4.71, and SD = 0.432. This shows that climatic traits, representing wind velocity, have a lower influence upon the main nutritional traits of donkey milk colostrum compared to environmental traits such as temperature and relative humidity, but nevertheless may influence the fat and protein content in donkey milk colostrum (Table 5).

At national policy levels, our research results may be of interest, not only in terms of emphasizing the environmental traits favorable for the nutritional composition valuation of donkey colostrum, but also in terms of a contribution towards enhancing sustainable agriculture. In support of this idea, we mention that the robustness of this species may permit the foundation of donkey farms in marginal areas with less soil fertility for the purposes of dairy production, as well as their use for promoting traditional tourism. Not least, we also mention that the revival of donkey rearing contributes to the preservation of biodiversity, considering the decreasing tendency of stocks in Romania, which, according to FAOSTAT data, recorded a total of 35,000 head in 1990, while in 2016 that number had decreased to 30,206 head.

Due to the limitations of this study, which only approaches the main nutritional components of donkey colostrum (fat, protein, and lactose), together with water content and pH, over a short-term, one-year postpartum period in spring, future research directions may be oriented toward long-term study, over several years, in both cold and warm postpartum seasons, as well as towards studying donkey milk colostrum content in terms of lactoferrin and β -lactoglobulin as a function of environmental conditions.

4. Conclusions

The results of our trial show that donkey colostrum has, to a large extent, similar content to human colostrum in terms of nutrients (fat, protein, and lactose). In the meantime, our study emphasizes a relatively constant fat and lactose content in donkey colostrum, while protein exhibits high variations between the first and the last day of production, with a linear increasing tendency. Strong positive, and strong to moderate correlations were identified between donkey colostrum nutrients, emphasizing their interrelationship. Our study makes an original contribution to the field of research concerning the possible influence of climatic factors on donkey colostrum content in nutrients. Thus, the PCA emphasizes three factors which influence the nutritional quality of donkey milk colostrum, labeled as: colostrum nutritional traits, environmental air traits and some colostrum nutritional traits, and climatic traits and some colostrum nutritional traits. Taking into account the benefits of donkey milk colostrum,

in terms of nutrients, as well as growth, immune, or anti-inflammatory factors, within a national and EU context that is characterized by a decrease of donkey stocks, further studies are needed in order to obtain detailed information concerning this natural source of health and the exogenous factors affecting its quality.

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