**Original Research Paper**

**Evaluation of Brix refractometry for the estimation of colostrum quality in jennies.**

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**Abstract**

Donkey placenta does not allow the passage of immunoglobulins, thus foals are born hypo-gammaglobulinemic and an adequate intake of high-quality colostrum in the first 24 hours of life is crucial for the surviving. The study aims to assess the relation between colostrum IgG concentration evaluated by the single radial immunodiffusion (SRID) test and the Brix refractometer in donkeys in order to establish a cut-off value for high quality of colostrum based on Brix refractometry. Colostrum was collected at foaling, and at 6, 12, 24 hours after foaling from the left and the right half of 9 Amiata jennies. A total of 72 colostrum samples were analyzed. A Friedman test with a Dunn’s test for multiple comparison was used for assessing the differences between the left and right half at each sampling time. No differences were found between the left and right halves; the average value was used to analyze the effect of sampling time on the IgG concentrations and Brix values. The relationship between colostrum IgG concentrations (SRID test) *vs* Brix value and Brix value *vs* time were analyzed using two different mixed linear models. A strong statistically significant relation has been found between IgG concentrations and Brix value (R2=0.84). The relation between IgG concentrations and Brix refractometer showed a cut-off point of 17% Brix score for the identification of high-quality colostrum. Brix value (%) decreased continuously from 16.29 by 0.29 x hour. Jennies and donkey foals’ management may be greatly improved using this simple and cheap device.

*Keywords:* Brix refractometer, Colostrum evaluation, Colostrum IgG, Jenny, Passive transfer.

**1. Introduction**

All species belonging to genus *Equus* are characterized bythe same typology of placenta, which is defined as diffuse, epitheliochorial and non-invasive [1]. Donkeys placenta, like other *Equus*, does not allow the passage of immunoglobulins from the jenny to the donkey foal, thus foals are born hypo-gammaglobulinemic. Foals’ survival strongly depends by normal foaling and normal vitality of the donkey foal; the Apgar score is a system that is commonly applied for the assessment of newborn vitality [2]. An adequate intake of high-quality colostrum in the first 24 hours of life plays also a crucial role in avoiding the development of the failure of passive immunity transfer (FPT) leading to a better survivor rate [2;3;4].

Mare colostrum is composed by a large amount of nutritional and immune components, most of which are represented by immunoglobulins G isotype (IgG). Colostrum quality usually refers to the quantity of IgG presented in a liter, it can be influenced by dam characteristics (breed, age of the animal, health status) and by different type of management and nutrition strategies [4;5]. In equine species, colostrum can be classified based on its quality as “very good”, if the IgG concentration is higher than 80 mg/mL, as “good quality”, if the IgG concentration is between 50 and 80 mg/mL, as “fair quality” if it shows IgG concentration between 28 and 50 mg/mL, or as “poor quality” when IgG concentration is less than 28 mg/mL [6]. In bovine, colostrum is classified as high quality when Brix value is higher than 21% Brix scale [7], while the equine colostrum is classified as high quality when colostrum is more than 23% Brix scale [8].

Due to the type of placentation and to the variation in colostrum composition is easy to understand why the quality of colostrum is so important for the foal’s life. The gold standard method for the estimation of IgG concentrations in the horse is the single radial immunodiffusion (SRID) test [9]. A strong homologue between horse and donkey IgG has been showed in literature [10;11]. SRID test needs about 24 hours to obtain the results; this delay decreases the usefulness of this test in the field because in case of FPT an early diagnosis for a prompt therapeutic intervention is mandatory [12]. Other qualitative techniques used to evaluate IgG colostrum concentrations in horses are the colostrometer [13] and the Brix refractometer [8;13;14]. The Brix refractometer is usually used for measuring the sucrose concentration in liquids such as fruit juice, molasses, and wine. When it is used for non-sucrose-containing liquids, percentage Brix (% Brix) approximates the total solids percentage [15]. The evaluation of the colostrum quality using the Brix refractometer is faster and easier to be performed in field compared to colostrometer. Brix refractometer may be the best method for the assessing of the colostrum quality also in donkeys and under field conditions. The aim of the present study was to evaluate the relation between colostrum IgG concentration evaluated by SRID and the Brix refractometer in the donkey colostrum and to establish a cut-off value for high quality of colostrum based on Brix refractometry.

**2.** **Materials and methods**

*2.1 Animals*

The present prospective study was approved by the Ethical Committee, University of Pisa (Organismo Preposto Benessere Animale, OPBA) (n.ro 22/19). A cohort of 9 Amiata jennies were enrolled in the present study. All the jennies owned to the Regional studfarm “Le Bandite di Scarlino” (Grosseto, Italy). An owner’s written consent was obtained.

All the jennies were admitted at the Veterinary Teaching Hospital “Mario Modenato”, Department of Veterinary Sciences, University of Pisa for late pregnancy and delivery monitoring during the 2019 foaling season. All the jennies underwent similar management conditions. In particular, mares were housed in collective paddocks (3 animals/each) during late pregnancy. Between 10 and 15 days before the presumptive delivery, jennies were housed in individual 6x6 m boxes where they were kept with their foals until the second week post-partum. Jennies were fed with grass hay *ad libitum* along with a commercial grain feed, according to the nutrient requirements stated by the NRC recommendations [16]. The average age of jennies was 12.11±4.07 years, while the average parity number was 4.62±2.34.

*2.2 Delivery management*

The mammary gland growth and calcium concentration in the mammary secretum were evaluated in order to assess the impending delivery [17]. When mammary secretion was present, the milk calcium concentration was evaluated every 24h at 6:00 pm using a commercial colorimetric kit (FoalWatch Titrets for Daytime Foaling Management, Chemetrics, Inc., Calverton, VA USA). Jennies’ 24-hours monitoring began when the calcium concentration was >200 ppm [18]. An operator visually supervised each phase of the foaling. In case of needs, the delivery was assisted.

*2.3 Inclusion Criteria*

Jennies were included in this study if the gestation length was between 339 and 370 days [19], if their delivery was unassisted and if they did not show any sign of premature lactation.

*2.4 Sampling procedures and Brix evaluation*

The udder of the jenny was washed with warm water and soap to remove debris and bacteria [6]. The first strip of colostrum from each half was throwed away. Then, 10 mL of colostrum were collected in a sterile milk tube 5 to 10 minutes after foaling (T0), and at 6 (T6), 12 (T12), 24 (T24) hours after foaling from the left (l) and the right (r) half by hand milking. Colostrum has always been collected before the foal’s nursing. Samples were divided into two aliquots. Colostrum from one of the two aliquots has been evaluated two times within 30 minutes after delivery by the same operator using an optical Brix refractometer (Atago brix N1, Japan) with a range of 0 to 32% Brix by putting a drop of colostrum on the Brix refractometer. The mean of the two different Brix evaluations for each colostrum sample has been recorded. The second aliquot of each colostrum sample was stored at -20° C until the SRID evaluation.

*2.5 Single Radial Immunodiffusion (SRID)*

IgG concentrations in colostrum were analyzed using a SRID assay specific for horse [10;11]. All the colostrum samples were analyzed SRID using a Horse IgG IDRing (R) Test (IDBiotech, France), in a single batch. Test results were determined by the comparison with a standard curve prepared using equine immunoglobulin standards supplied with the Kit. Samples were diluted 1/600 before IgG determination because of their high IgG concentrations.

*2.6 Statistical methods*

A Friedman test with a Dunn’s test for multiple comparison was used to evaluate the differences between the colostrum IgG concentration and the Brix value for the left and right half at each sampling times. Since no statistically significant differences were found, both for IgG and Brix measurements, between the left and right halves, the average value between those two was used to analyze the effect of sampling time on the IgG concentrations and Brix values.

Data concerning colostrum IgG concentrations (as average of left and right half) at different sampling times were evaluated for distribution using a Shapiro-Wilk test. Since data showed a non-Gaussian distribution, a Log10 transformation was applied to normalize the distribution.

The results about colostrum IgG concentrations were analyzed with the following regression model and expressed as mean±standard error.

Yij= m + b Brix + Animalj + eij

Where: Y is the individual value of colostrum IgG for each jenny; Brix is the continuous effect of Brix value at the same sampling time, b is the regression coefficient; Animal is the random jenny effect (j = 1 to 9); and e is the random error.

Using the same formula, a cut-off value for Brix analysis of the colostrum quality can be assessed by the given IgG concentration reported for high quality colostrum in horses (80 mg/ml) [6].

In order to assess the variation of colostrum Brix value during the first 24 h after birth, Brix value was evaluated according to the following mixed model:

Yijk= m + Timek + Animalj + eijk

Where: Y is the Brix value for each Jenny; Time is the fixed effect of the time of sampling after birth of the donkey foal (0h, 6h, 12h, 24h); Animal is the random donkey foal effect (j = 1 to 9); and e is the random error.

Statistical significance was set at 0.05. Statistical analysis was performed using a commercial software (Graph Pad Prism, 6.0, USA)

**3. Results**

No jennies were excluded from the study due to gestation length or premature lactation. The average data concerning the 72 colostrum samples produced by nine jennies at different sampling times entered in the statistical model. Results concerning colostrum evaluation made by SRID and Brix analysis at each sampling time were reported as mean ± standard deviation in Table 1.

A strong significant relation between IgG concentrations and Brix value of colostrum (p<0.0001, R2=0.84) were found (Graph 1). The value of colostrum IgG can be calculated using a given Brix value with the formula: *Log10 IgG (mg/mL) = 0.74 + 0.07 x Brix value (%).*

Based on the above formula, colostrum could be classified as “very good” if the Brix value is higher than 17%, “good” if the Brix value is between 14 and 17%, “fair” when the Brix value is between 10 and 14% and “poor” when the Brix value was below 10%.

A significant relation between Brix value of colostrum and time (p<0.0001, R2=0.58) has been found (Graph 2). Brix value (%) decreased continuously in all colostrum samples from 16.29 by 0.29 x hour.

**4. Discussion**

Literature concerning the quality of donkey colostrum in terms of immunoglobulin concentrations is still poor but the possibility for an easy assessment of colostrum quality in donkeys is essential for the foal to avoid FPT and the relative pathological *sequelae*. The aim of the present study was to evaluate the relation between colostrum IgG concentration evaluated by SRID and the Brix refractometer in the donkey colostrum and to establish a cut-off value for high quality of colostrum based on Brix colostrometry.

Studies reported a close similarities of the immune systems of horses and donkeys, thus, due to the lack of a specific SRID for donkey, the IgG concentration of the jennies’ colostrum were evaluated using a SRID assay specific for horses [10;11]. All the jennies included in this study showed a good or very good quality of colostrum at T0 [6]. Colostrum IgG concentration did not differ between the left and the right half at any sampling time, in line with what reported by Doreau and colleagues [20] for horses. This finding may mean that colostrum does not need to be collected by both halves for assessing the quality, instead it could be checked just by one half. Thus, the colostrum quality evaluation may be easier and faster to achieve even for an owner, or in breeding farms with a high number of animals.

In this study, the Brix value was significantly related with the colostrum IgG concentration (R2=0.84) in the first 24 h after foaling, which confirms the result obtained in previous studies in in horses (R2=0.94; R2=0.93) [13;14]. Moreover, using the formula provided in the present study, the exact amount of colostrum IgG concentration can be estimated from the given Brix value. This could be very useful especially in field condition.

The cut-off value indicating a high-quality colostrum in donkeys was 17% of the Brix scale. This value was lower compared to bovine and equine species in which were 21% and 23%, respectively [7;8]. This difference may be related to the species. Also, the present study showed preliminary data with a restricted population; increasing the number of animals may be indicated for further studies.

Colostrum Brix values were statistically higher immediately after foaling compared to 12 and 24 h after foaling, and values tend to decrease continuously by 0.29 % every hour. This could be due to the normal physiological transition between colostrum and milk production that lead to a rapid decreasing in IgG concentrations especially during the first 24 hours after foaling. Our results are in line with what reported in horses in which colostrum IgG concentration statistically reduced from a value of 54.5 mg/mL obtained during the first 4 hours after foaling, to 10.1 mg/mL between 9 to 12 hours postpartum [21]. Our results were also in line with Veronesi and colleagues [22] who found no statistically significant differences in the IgG colostrum concentration collected from jennies in the first 24 hours after foaling, but there still a trend of decreasing in IgG concentration in the first 12 hours (from 29.5 to 16.3 mg/dl). Moreover, the Authors reported lower IgG concentration values compared to our study. These differences might be due to breed differences, to the different management of colostrum sampling and to the different type of analysis performed to the samples. In the previous study [22], colostrum was collected several days before parturition, thus it can be possible that the quality of colostrum was influenced by the sampling method.

Colostrum Brix values at T0 and T6 did not show any statistically significant difference, while colostrum IgG concentration evaluated at T0 and T6 by the SRDI analysis was significantly different. This dissimilarity may be due to the higher sensibility of the SRID test which is considered the gold standard test for colostrum evaluation in equids. Moreover, this may be influenced by the moderate relation between colostrum Brix value and time found. Despite this, the colostrum Brix assessment was significantly related with the colostrum IgG concentration in the first 24 h after foaling, as previously discussed. The colostrum Brix evaluation can be strongly suggested as a feasible analysis in donkeys, especially under field condition as reported for other species. High quality colostrum, evaluated by Brix refractometer, could be frozen and used in case of jenny with low quality colostrum or in orphan donkey foal.

Finally, in further studies it would be interesting to assess the potential relation between colostrum IgG values, Brix colostrum and birth weight in donkeys. In equine, foals need a minimum of 1.0–1.25 g IgG/kg bodyweight to achieve serum IgG above 8 g/L and also in calves the absorption is related to the calf bodyweight [23;24].

**5. Conclusion**

Jennies and donkey foals’ management may be greatly improved by using Brix refractometer technology. The present preliminary data seems to suggest that an appropriate cut-off level for colostrum Brix evaluation is equal, or above, to 17% in order to ensure a high quality colostrum. This simple and cheap device could allow practitioner to assess the colostrum quality immediately after foaling to avoid the risk of FPT in the donkey foal.

**Authorship**

LT – acquisition of data, drafting and revising the article, final approval of the version to be submitted; IN, FB – acquisition of data; MM – analysis and interpretation of data; MS – design of the study, acquisition of data, interpretation of data, revising the article, final approval of the version to be submitted.

**Declarations of interest**

None.

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**Figure captions**

Graph.1. Relation between IgG colostrum concentrations (as Log10) and Brix value at different sampling time (P<0.0001) R2 = 0.84.

Graph.2. Relation between Brix value and time throughout the first 24 hours after foaling in a population of Amiata jennies (P<0.0001) R2 = 0.58.

**Tables**

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| --- | --- | --- | --- | --- |
| Colostrum evaluation | T0 | T6 | T12 | T24 |
| IgG (mg/mL) | 1.95±1.42a | 1.76±1.49b | 1.56±1.17c | 1.40±0.97c |
| Brix value (%) | 17.58±2.86d | 13.86±2.36d | 11.13±1.77e | 10.16±1.06e |

**Table 1. Colostrum IgG concentrations (as Log10) and Brix value, expressed in mean and standard deviation, evaluate in a population of 9 jennies for the average colostrum value of right and left halves at different collection time. Legend: T0 – after birth; T6 – 6 hours after birth; T 12 – 12 hours after birth; T24 – 24 hours after birth. Legend: a≠b≠c; d≠e p < 0.05.**