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SHORT COMMUNICATION

Rapid and Accurate Assay to Detect IgG Concentration in Colostrum of Dezhou Donkeys

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A B S T R A C T

High-quality colostrum is crucial in preventing hypogammaglobulinemia and infectious diseases in foals. This study aims to estimate the suitability of a Brix refractometer for estimating IgG concentration. To estimate the reliability of using the refraction method (Brix refractometer) to assess colostrum quality in Dezhou donkeys by comparing its performance with that of the radial immunodiffusion method. Colostrum samples (n=69) were collected from 23 Dezhou jennies at 0, 6, and 12 hours after birth. Regression analysis and one-way analysis of variance (ANOVA) were carried out on the collected data. The results showed strong correlations between the two methods ($P<0.01$). Regression analysis revealed a statistically significant relationship between the IgG concentration and Brix value ($R^2=0.9177$). By using the regression equation between the two methods, the colostrum of Dezhou donkeys could be classified into four categories: excellent, good, fair, and poor quality, based on Brix values greater than 17.96, between 15.03 and 17.96, between 12.88 and 15.03, and less than 12.88%, respectively. These results suggested that the refraction method can be a reliable and efficient alternative to estimate colostrum quality in Dezhou donkeys.

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INTRODUCTION

The continued growth of China's economy has increased disposable income for its people, enabling them to spend more on personal well-being and associated health products. This has caused renewed interest in Traditional Chinese Medicine and a surge in demand for compounds such as Ejiao, which is produced from donkey skin and has been used since ancient times to treat blood disorders plus reproductive problems (Kubo and Zhao, 2022; Yu *et al.*, 2025). Unfortunately, the popularity of Ejiao in China caused a ten-fold increase in donkey prices worldwide and promoted widespread illegal trafficking in many countries (Waters, 2019; Bennett and Pfuderer, 2020). The Chinese government issued a policy decision in 2014 encouraging intensive donkey farming to combat Ejiao deficiency (Seyiti and Kelimu, 2021; Zhang *et al.*, 2023). Consequently, numerous intensive donkey farms were established in China, the largest of which houses over 10,000 animals. However, mortality in foals on some farms can be more than 30% due to infectious diseases, due to the failure of passive immune transfer (FPIT), which is mainly caused by poor colostrum quality.

Donkeys and horses possess similar structures of placentas with a six-layer placental barrier, which hinders the transfer of immunoglobulins to the foal and makes the foal hypogammaglobulinemic when born (Carluccio *et al.*, 2008). Therefore, foals need a timely intake of good-quality colostrum to gain immunity and prevent infections in the first few months of life. Donkey colostrum contains antibodies and various nutrients, such as immunoglobulins, lactoferricin, lysozyme, and peptides, which play crucial roles in activation of innate immune system essential and nourishment for foals to protect them

against infectious diseases in the first few months after birth (Li *et al.*, 2021; Ning *et al.*, 2023). Among them, immunoglobulins, including IgG, IgA, and IgM, are critical components in colostrum, with IgG accounting for over 70% of the total immunoglobulin content. Therefore, the level of IgG is directly related to the colostrum quality (Schneider and Wehrend, 2019; Gonçalves *et al.*, 2024). In addition, the colostrum quality is also affected by the breed, age, environment, feeding factors, health status, and many other factors (Coverdale *et al.*, 2015).

Several methods have been developed for IgG estimation, such as radial immunodiffusion test (gold standard), automatic turbidimetric immunoassay, glutaraldehyde coagulation (GC) test, refractometry, and enzyme-linked immunosorbent assay (ELISA), with each having its advantages and disadvantages (Clabough *et al.*, 1989; Ujvari *et al.*, 2017). Unfortunately, many of these methods are relatively slow and require more than 24 hours to provide the results. Therefore, there is an urgent need for rapid and accurate methods that enable the field assessment of colostrum quality during critical feeding periods. A Brix refractometer was initially used to estimate sucrose content in solutions. In recent years, it has been widely used to assess colostrum quality and passive immune transfer in neonatal foals, owing to its ability to detect soluble solids in solutions Turini *et al.*, 2020b; Turini *et al.*, 2021; Felix *et al.*, 2022). However, there has been no report on assessing colostrum quality using a Brix refractometer in Dezhou donkeys.

The Brix value of colostrum varies significantly between species (e.g., cattle, sheep, etc.), and the quality of colostrum can also differ significantly among donkey breeds (Bartier *et al.*, 2015; Turini *et al.*, 2020a; Turini *et al.*, 2020b; Felix *et al.*, 2022; Hamer *et al.*, 2023). Therefore, it is essential to establish specific parameters for each breed. The Dezhou donkey, one of China's five most popular donkey breeds, is a primary breed in large-scale donkey farms. Hence, this study attempted to evaluate the colostrum quality of Dezhou donkeys using a Brix refractometer under large-scale breeding conditions, aiming to compare the refraction method and radial immunodiffusion method in assessing the colostrum quality of Dezhou donkeys and establish Brix values for estimating the colostrum quality based on Brix refractometer.

MATERIALS AND METHODS

Ethical approval: The Animal Protection Association, Qingdao Agricultural University, approved the study. It was conducted according to local legislation and institutional requirements.

Animals: This study involved 23 healthy Dezhou jennies, who were equally managed and were 3 to 7 years old. Their health was evaluated as BSC-3 per the body condition score (BCS) system.

Breeding and management of pregnant jennies: In the first 11 months of gestation, the jennies were kept in standard enclosures, while in the last month of gestation, they were kept in a straw-covered maternity paddock for adaptation to the delivery site. Two meals were fed daily at 8:30–9:00am and 2:30–3:00pm. Each animal was offered 1.25kg of concentrate and 5.5kg of hay (comprising 1.25kg of wheat straw, 1.75kg of bean straw, and 2.5kg of peanut seedlings) per meal, along with ad libitum access to water and a mineral lick.

Delivery management: A preliminary delivery date was estimated based on the gestation period (348–377 days), and the mammary gland and vulva were closely monitored during the final month. When signs such as redness of the mammary gland and vulva, restlessness, decreased appetite, heavy respiration, and frequent abdominal checking were observed, overnight surveillance from a distance was implemented to provide necessary assistance during parturition.

Colostrum collection: Sixty-nine samples were collected during the parturition season, spanning from spring to autumn. Three samples were collected from each Jenny at 0, 6, and 12h after delivery. About 15mL of samples were collected in sterile tubes, and the IgG concentration was determined. The left colostrum samples were stored at -20°C for subsequent analyses.

Radial immunodiffusion test (Gold standard): The IgG concentration in the colostrum of jennies was determined using the Equine IgG Test Radial Immunodiffusion Test Kit and preliminarily estimated based on the Brix value of the colostrum. Colostrum samples with IgG concentrations were likely to perform better than the manufacturer's stated performance for the assay (>3000mg/dL), and they were diluted ten-fold with normal saline. Then, 5μL of the sample was added to the plate wells and incubated at room temperature (22–24°C) for 48h, and the diameter of the precipitation ring was measured using a vernier caliper. The average of three replicates of the assay standards was used to construct a calibration curve of the IgG concentration in colostrum samples.

Brix refractometer estimation of colostrum: After collecting colostrum, the quality was promptly estimated using an optical Brix refractometer (Tianyuan®, China) with a Brix value range of 0–32% by putting three drops of colostrum on the prism. The automatically generated value was recorded. Each sample was measured in parallel three times, and the average was recorded. All samples were measured under air conditioning at 25°C. Before measuring each new sample, the refractometer was cleaned and calibrated with ultra-pure water.

Statistical analysis: Regression analysis and one-way analysis of variance (ANOVA) were used to assess the statistical differences via the GraphPad Prism 9.0 (GraphPad Software, La Jolla, CA). Correlation analysis was performed with SPSS 27.0. Data were presented as mean±SD. $P<0.05$ and $P<0.01$ indicated significant correlations. The asterisks *, **, ***, and **** indicate $P<0.05$, 0.01, 0.001, and 0.0001, respectively.

RESULTS AND DISCUSSION

Foals are born with naive immune defenses because they do not possess a fully developed immune system and

do not receive any antibodies from their dam while in the uterus (Aoki *et al.*, 2020; Wang *et al*., 2024; Smith *et al*., 2025). They rely on a passive transfer of antibodies from their dam to help build their immune defenses. Therefore, consuming enough high-quality colostrum in the first several hours is essential for their health. However, almost no donkey farms currently evaluate the colostrum quality after mare delivery in intensive donkey farms in China; the main reason is that there is no convenient tool to determine colostrum quality. The refraction method has been successfully used in dairy farms (Bielmann *et al.*, 2010; Quigley *et al.*, 2013; Zhou *et al.*, 2023a; 2023b), so it is necessary to verify its reliability for determination in donkey colostrum. The results of the present study suggested that the refraction method can be used as a reliable and efficient method to estimate colostrum quality in Dezhou donkeys.

From a veterinary perspective, the definition of good-quality colostrum is characterized by a high concentration of IgG (Rampacci *et al.*, 2023; Stahl *et al.*, 2024; Kayasaki *et al*., 2025). According to literature related to equine species, colostrum is considered excellent, good, fair, and poor at IgG concentrations >8.0, 5.0–8.0, 2.8–5.0, and <2.8g/dL, respectively. A radial immunodiffusion test, considered the gold standard, can determine the exact concentration of IgG in colostrum. Although other nutrients except IgG can affect the value of the Brix refractometer, we can use the result of Radial immunodiffusion test (gold standard) to estimate the reliability of using Brix refractometer for the assessment of colostrum quality, which is the purpose of this study. Fortunately, the results showed strong correlations between the two methods ($P<0.01$). Regression analysis revealed a statistically significant relationship between the IgG concentration and Brix value ($R^2=0.9177$). These results suggest that the refraction method can be used as a reliable and efficient method to estimate colostrum quality in Dezhou donkeys. The colostrum Brix value significantly decreased ($P<0.05$) 6 hours after delivery. It further significantly declined to 12h relative to 0h after delivery (Fig. 1). The same results were obtained for IgG concentrations in colostrum as measured by radial immunodiffusion. The results of the present study indicated that the colostrum of Dezhou donkeys has excellent quality at 0h after delivery, with an average IgG concentration of 9.45g/dL as measured by the radial immunodiffusion test (gold standard). However, there were three jennies with poor-quality colostrum. At 12h after delivery, the IgG concentration in the colostrum significantly decreased to 1.61g/dL (Fig. 2). Turini et al. reported that the average IgG concentration in Amiata donkey is 1.95g/dL immediately after delivery (Turini *et al.*, 2020b); however, Felix *et al.* (2022) found that the IgG concentration in colostrum of Nordestino ecotype donkeys is, on averagely 8.71g/dL immediately after delivery (Felix *et al.*, 2022). The average IgG concentration in Dezhou donkeys detected in this study (9.45g/dL) was significantly higher than previously recorded values, indicating that colostrum quality varies substantially among donkey breeds. Therefore, it is highly necessary to establish specific parameters for different breeds of donkeys.

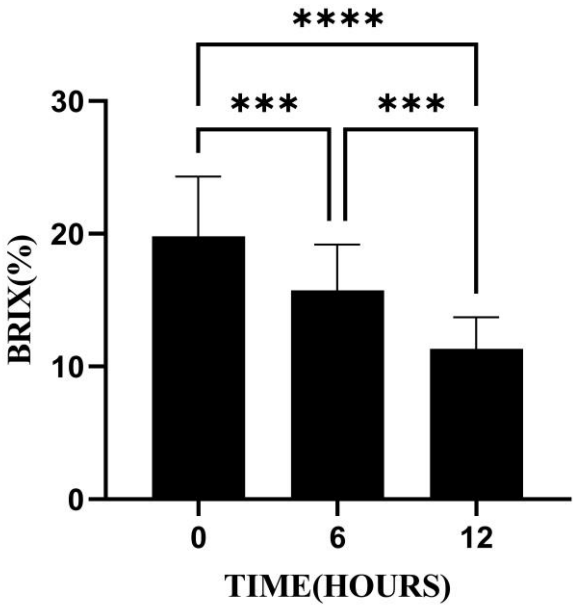

Fig. 1: Differences in Brix values of Dezhou donkeys' colostrum at various times.

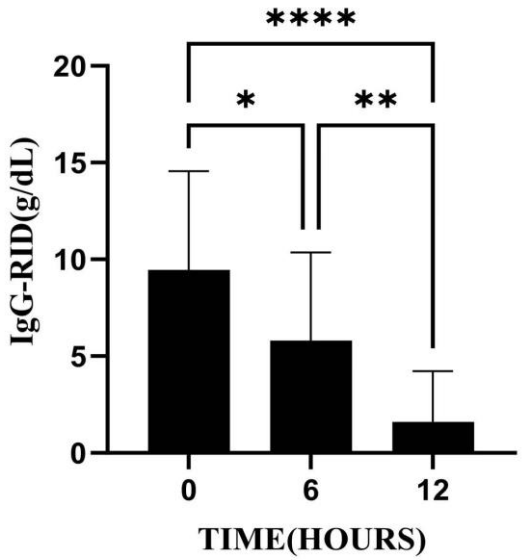

Fig. 2: Differences in IgG concentration in Dezhou donkey colostrum at various times.

The IgG concentration measured by radial immunodiffusion showed a similar trend to the Brix value estimated via refractometer, with the highest concentration at 0h after delivery, which showed a consistently decreasing trend thereafter (Fig. 3). Our results showed that the change in colostrum quality was significantly different within 12h and exhibited a consistently declining trend, which is similar to the results reported by Felix *et al*. (2022). Therefore, the foal must ingest high-quality colostrum as soon as possible to ensure the successful transfer of passive immunity.

Importantly, we found a strong correlation between the IgG concentration measured by radial immunodiffusion and the colostrum Brix value measured by refractometer ($P<0.01$) (Fig. 4). According to the relevant literature and the regression equation between the radial immunodiffusion and refractometer method, the colostrum of Dezhou

donkeys can be classified excellent, good, fair, and poor quality with Brix values >17.96, 15.03-17.96, 12.88-15.03, and <12.88%, respectively (Fig. 4).

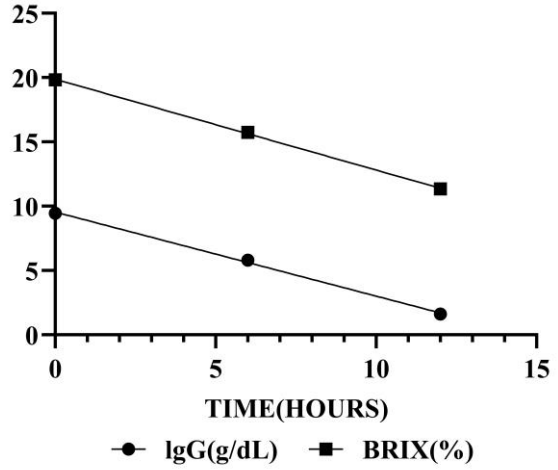

Fig. 3: The IgG concentration was measured using the radial immunodiffusion method, and the Brix value was measured using the refractometer method, both of which changed over time. Regressions: IgG RID ($y=-0.007681x^2-0.5614x+9.453$; $R^2=0.3749$), BRIX ($y=-0.004589x^2-0.6514x+19.82$; $R^2=0.4984$).

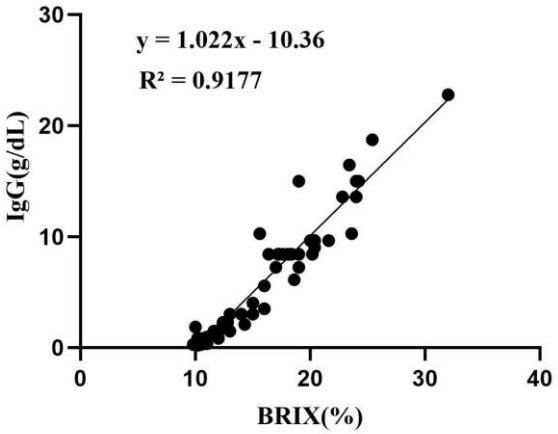

Fig. 4: Relationship between IgG concentrations in colostrum in Dezhou donkey by radial immunodiffusion and Brix value by refraction.

Conclusions: The present study demonstrated a strong correlation between the IgG concentration in colostrum, as measured by the radial immunodiffusion test, and the Brix value, as measured by a refractometer. Additionally, these two methods produced consistent results in other aspects. Therefore, the Brix refractometer can serve as a safe and efficient tool for evaluating the quality of Dezhou donkey colostrum, ensuring timely intake of high-quality colostrum by foals and preventing the failure of passive immunity.

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Conflict of interest: None.

Authors contribution: JD and YT conceived and designed the study. CL, NG, ZD, HL, ML, CW, HW, JY, TW, YG, CH, KZ, YC, MW, WZ, WZ, YT, and JD executed the experiment. CL was involved in data analysis and writing. AK edited the manuscript. All authors approved the final version of the manuscript.

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