



## Research Paper

## Assessment of Passive Immunity Transfer in Indigenous Algerian Cows

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## ABSTRACT

**Introduction:** The acquisition of passive immunity is essential for calf survival, as they are born agammaglobulinemic and must absorb maternal antibodies from colostrum. This immunity is especially fragile in calves from extensive farming systems where indigenous cattle face environmental challenges. The objective of the current study was to determine the prevalence of sufficient passive immunity transfer (PIT) in order to assess neonatal survival capacity under natural environmental conditions.

**Materials & Methods:** For this purpose, 62 blood samples were taken from the jugular vein of local calves born in the mountainous regions of northeastern Algeria, to measure serum total proteins (STPs) levels using a digital refractometer.

**Results:** The Mean±SD of STP concentration in calves was 18.56±15 g/L, and the Mean±SD serum Brix percentage was 8.8±1.3%. STP correlated strongly with% Brix ( $r=1$ ). 58% of calves presented a STP concentrations >10 g/L, showing sufficient transfer of passive immunity and 42% had a STP concentrations <10 g/L, indicating failure of passive transfer (FPT). Our study revealed that the rate of PIT was influenced by the calving score ( $P=0.03$ ). However, the sex of the calf had no impact on the success of this transfer ( $P=0.07$ ). Test characteristics were evaluated to assess the FPT determined as STP level below 10 g/L. Receiver operating characteristic (ROC) curve analysis identified a best cutoff value of 8.4% Brix, which indicated a sensitivity of 89% and a specificity of 85%. Our results are compared with published data, considering the methods and populations studied.

**Conclusion:** Farmers can use the digital refractometer to measure STP in calves to facilitate monitoring the success of passive transfer to calves born in mountainous regions with free-range grazing.

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## 1. Introduction

The transfer of passive immunity through colostrum is essential for the health and proper development of newborn calves [1, 2]. Colostrum, the first postpartum mammary secretion, is rich in immunoglobulins, nutrients and bioactive factors that provide immunological protection and nutritional support to bovine neonates [3, 4]. Calves are born almost agammaglobulinemic with a complete but immature immune system. They acquire passive immunity after birth by absorbing immune-related cells and proteins, primarily IgG, from colostrum through the gut.

Local Algerian cows are well adapted to their natural environment, which facilitates calving. Their slightly inclined pelvis and wider pelvic opening ease the calf's passage during birth, significantly reducing the risk of obstetrical complications. Thanks to these traits, the cows calve naturally without human intervention, ideal for a free-range grazing system. After birth, they effectively nurse their calves, ensuring a good start for the newborn. This autonomy supports a more rustic husbandry style, less dependent on constant farmer supervision [5].

Colostrum should be given to the calf immediately after birth, ideally within the first 4 hours, to maximize immunoglobulin absorption for disease protection. The intestine's ability to absorb immunoglobulins declines rapidly over time; each hour of delay in feeding the first colostrum increases the risk of failure in transferring immunity by 13% [6, 7].

Immunoglobulin absorption is most efficient within the first 24 hours of life, declining sharply thereafter. Failure to transfer passive immunity (FPT) is associated with increase calf morbidity and mortality, bovine respiratory disease, neonatal diarrhea, and reduced productive performance later in life [1, 6, 7].

Calves are considered immunologically protected when their serum concentration of immunoglobulin G (IgG) exceeds 10 g/L. FPT significantly rises the risk of morbidity and impaired growth in neonatal calves [8].

Several tests are commonly used to evaluate colostrum quality [9]. The radial immunodiffusion (RID) assay is the gold standard for measuring serum IgG levels to determine passive transfer; however, it requires laboratory technicians and takes 18 to 24 hours, making it impractical for farm use [10].

Indirect tests, such as Brix refractometry [11], offer a cost-effective, on-site alternative for measuring serum total protein (STP) concentration and estimating IgG in colostrum. STP evaluation via refractometry is inexpensive, easy to use, and suitable for field application [12]. Brix degrees correlate highly ( $r=0.87$  to  $0.93$ ) with serum IgG concentration [10, 13]. Calves with IgG levels above 10 g/L are considered protected; FPT presence correlates with health and performance issues [8]. Thus, STP evaluation can be used on farms to monitor colostrum management and predict calf health.

The objective of this study was to evaluate the efficacy of passive immunity transfer (PIT) by measuring total calf serum protein concentrations using a digital refractometer, to estimate the ability of indigenous calves to survive after birth in difficult environmental conditions.

## 2. Materiel and Methods

### 2.1. Assessment of passive immunity transfer

The study was conducted from February 2023 to September 2024, in the wilaya of Guelma, situated in northeastern Algeria. This area represents the origin of the indigenous cattle breed known as the "Guelmoise." Owing to its strategic geographic position, favorable climatic conditions, and the presence of locally adapted cattle breeds, Guelma serves as a critical center for the advancement of the livestock sector, particularly focusing on native cattle breeds characterized by their resilience and adaptability to challenging environmental conditions.

After calving, blood samples were obtained from 62 local calves aged between 1 and 5 by jugular venipuncture, using pure blood in a dry sterile tube without anticoagulant. The blood samples were returned to the laboratory and centrifuged. Serum was separated by centrifugation ( $3,500\times g$  for 10 min at  $20^{\circ}\text{C}$ ) and collected to estimate passive transfer of immunity using refractance on a Brix scale with a standard digital refractometer (MC2-1 HS90275000) with automatic temperature compensation ( $10$  to  $50^{\circ}\text{C}$ ), a resolution of  $0.1\%$  Brix, and a range of  $0$  to  $50\%$  Brix. The refractometer was calibrated with distilled water at the start of each set of analyses. The %Brix value will be converted to g/L according to the Calf Note 214 conversion table [10, 13–15]. Each calf was categorized as having adequate transfer of passive immunity when Brix percentage was  $\geq 8.4\%$  [10].

Total serum protein concentration was measured exclusively using a digital refractometer because of its rapidity, low cost, ease of use under field conditions, and its well-documented correlation with serum IgG concentration measured by the reference method, radial immunodiffusion ( $r=0.87$  to  $0.93$ ). This method is widely accepted as a reliable screening tool for evaluating PIT in calves, making the routine use of the reference method, unnecessary in large-scale field studies [10, 11, 14, 15].

## 2.2. The statistical processing of data was generated with Excel Stat 2014

The data were analyzed using the Student's t-test to compare means between two groups. When multiple groups were compared, analysis of variance (ANOVA) was used to detect significant differences between means. In cases of significant results, post hoc tests were performed to identify which groups differed from each other. The threshold for statistical significance was set at  $P<0.05$ .

The diagnostic performance of the test for assessing PIT in calves was evaluated by calculating sensitivity and specificity with 95% confidence intervals from  $2\times 2$  contingency tables, using serum IgG concentration as the reference standard. Receiver operating characteristic (ROC) curve analysis was also performed to determine the optimal diagnostic cutoff.

Sensitivity and specificity were estimated using previously validated total serum protein thresholds to classify calves as having adequate or inadequate PIT. As radial immunodiffusion was not performed, these values represent threshold-based performance estimates rather than true diagnostic accuracy measures relative to a reference standard.

## 3. Results

To the best of our knowledge, this is the first study conducted to estimate the PIT in the indigenous cattle population in Algeria.

In this study, the Mean $\pm$ SD of STP concentration was  $18.56\pm 15$  g/L; range 1.2–55.5 g/L, and the Mean $\pm$ SD Brix percentage was  $8.8\pm 1.3\%$ ; range 7.2–12.1%). Descriptive statistics for the serum samples are shown in Table 1.

The calves were classified by sex, with 38 males (61%) and 24 females (39%). In male calves, the Mean $\pm$ SD STP concentration was  $17.98\pm 12.79$  g/L (range: 1.2–55.5 g/L), and the Mean $\pm$ SD %Brix value was  $8.7\pm 1.15\%$ ; range: 7.2–12.1%). In female calves, the mean STP was  $20.11\pm 10.12$  g/L; range: 4.3–42.1 g/L, and the Mean $\pm$ SD %Brix was  $8.9\pm 0.91\%$ ; range: 7.5–10.9%).

Based on calving conditions, 60% of calves were born without assistance (score 1), 32% required mild assistance (score 2), and 8% required mechanical assistance (score 3). One case of neonatal mortality following cesarean delivery was recorded and excluded from the analysis. The Mean $\pm$ SD STP concentrations for calves with scores 1, 2, and 3 were  $27.5\pm 9.68$  g/L,  $5.6\pm 1.51$  g/L, and  $2.88\pm 0.99$  g/L, respectively.

The evolution of STP concentrations according to calf age (in days) indicate that on day 1 ( $n=8$ ), calves exhibited the highest Mean $\pm$ SD STP value ( $44.72\pm 5.5$  g/L), indicating effective passive transfer of immunoglobulins shortly after colostrum intake. A marked decline was observed on day 2 ( $n=15$ ), with Mean $\pm$ SD STP decreasing to  $23.8\pm 6.9$  g/L, followed by a further reduction on day 3 ( $n=18$ ;  $18.45\pm 7.7$  g/L). From day 4 onward, STP concentrations decreased sharply, reaching  $5.87\pm 1.23$  g/L on day 4 ( $n=12$ ) and  $3.51\pm 0.92$  g/L on day 5 ( $n=9$ ). This progressive decline indicates a rapid reduction in circulating immunoglobulins and total protein after the initial colostrum absorption period.

Precise information regarding the timing of first colostrum ingestion was unavailable. This was mainly due to the extensive husbandry system in mountainous areas, where calves are born on pasture and remain with their dams, preventing direct observation and accurate recording of colostrum intake. Such management practices are characteristic of indigenous cattle populations and reflect real-world production conditions. Nevertheless, serum total protein concentration was used as an objective and widely accepted indicator of PIT under field conditions.

Analysis of variance (ANOVA) of our data indicated that the type of calving influenced blood levels of STP, and consequently the success of PIT in the calf ( $P<0.05$ ). Highly significant differences were observed between calving scores 1 and 2, and between scores 1 and 3, with 95% confidence intervals (CIs) of 17.28%–25.5%, and 18.1%–30.98%, respectively. In contrast, no statistically significant difference was found between scores 2 and 3 (95% CI, -0.92%, 7.22%).

**Table 1.** Descriptive statistics of calf serum sample measurements

| Characteristic    | No. (%)              | STP (g/L) Brix% |             |           |      |         |      |
|-------------------|----------------------|-----------------|-------------|-----------|------|---------|------|
|                   |                      | Mean±SD         |             | Minimum   |      | Maximum |      |
| Total             | 62                   | 18.56±15        | 8.8±1.3     | 1.2       | 7.2  | 55.5    | 12.1 |
| Sex of calf       | Male                 | 38(61)          | 17.98±12.79 | 8.7±1.15  | 1.2  | 7.2     | 55.5 |
|                   | Female               | 24(39)          | 20.11±10.12 | 8.9±0.91  | 4.3  | 7.5     | 42.1 |
| Type of calving   | <sup>1</sup> Score 1 | 37(60)          | 27.5±9.68   | 9.6±0.87  | 5.18 | 7.6     | 55.5 |
|                   | <sup>2</sup> Score 2 | 20(32)          | 5.6±1.51    | 7.6±0.13  | 3.41 | 7.4     | 11.2 |
|                   | <sup>3</sup> Score 3 | 5(8)            | 2.88±0.99   | 7.35±0.09 | 1.2  | 7.2     | 4.52 |
| Age of calf (day) | 1                    | 8               | 44.72±5.5   | 11.13±0.5 | 34.4 | 10.2    | 55.5 |
|                   | 2                    | 15              | 23.8±6.9    | 9.24±0.6  | 11.2 | 8.1     | 36.6 |
|                   | 3                    | 18              | 18.45±7.7   | 8.76±0.7  | 5.2  | 7.56    | 37.7 |
|                   | 4                    | 12              | 5.87±1.23   | 7.62±0.11 | 3.41 | 7.4     | 8.18 |
|                   | 5                    | 9               | 3.51±0.92   | 7.4±0.08  | 1.2  | 7.2     | 4.74 |

<sup>1</sup> Unassisted (without any human intervention), <sup>2</sup> Easy help (one-person assistance, no mechanical aids), <sup>3</sup> Difficult, assistance from more than one person, or use of mechanical means.

In this study, forty-two percent of samples were classified as «poor TPI» with STP concentrations <10 g/L (Brix <8.4%), indicating FTPI (Figure 1).

58% presented a sufficient transfer of passive immunity with STP concentrations higher than 10 g/L. Among these calves, 19% were classified as «fair TPI» with a total serum proteins level between 10 and 20 g/L corresponding to %Brix 8.4 - 9.4%, while 39% have a level higher than 20 g/L with Brix >9.4% (classified as «excellent TPI»).

The percentage of male calves with a STP concentration higher and lower than 10 g/L was 53% and 47% respectively. About females, this percentage was 68% and 32% respectively. We determined that the sex of the calf had no significant effect on the total serum protein level ( $P=0.07$ ) with 95% CI [-5.92%, 11.69%].

### 3.1. Correlation

Correlation analysis showed that STP levels were strongly positively correlated with %Brix ( $r=1$ ) (Figure 2).

### 3.2. Test characteristics

The absence of a gold standard method, such as radial immunodiffusion, limits the interpretation of the calcu-

lated sensitivity and specificity. Therefore, these estimates should be interpreted with caution.

Test characteristics of sensitivity and specificity considered for the consecutive cutoff levels of 7.8 to 9% Brix, was determined for the assessment of FTPI (STP<10 g/L). The sensitivity and specificity for %Brix at previously recommended values are shown in Table 2.

A ROC curve was generated to plot the true positive rate against the false positive rate for %Brix values at 0.1 percentage unit increments, ranging from 7.3% to 8.8% Brix (Figure 3). The optimal combination between sensitivity (89%) and specificity (85%) was identified at a threshold of 8.4% Brix.

## 4. Discussion

In the mountainous regions of northeastern Algeria, extensive livestock farming relies on pasture-based systems. This necessitates the effective transfer of passive immunity to newborn calves through absorption maternal immunoglobulin from colostrum, thereby preventing neonatal mortality and reducing the risk of diseases associated with transfer failure. The use of a digital refractometer enabled assessment of total serum protein concentration to estimate the success of PIT, a key component of calf monitoring programs.

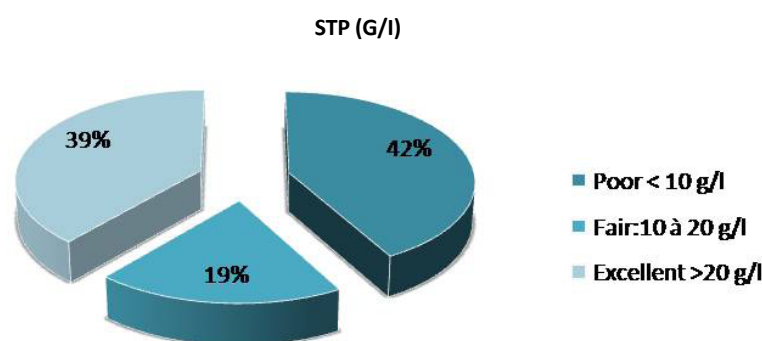


**Figure 1.** Percent of PIT

In the current study, the Mean $\pm$ SD STP was 18.56 $\pm$ 15 g/L with a range from 1.2 to 55.5 g/L, and the Mean $\pm$ SD %Brix was 8.8 $\pm$ 1.3% with a range from 7.2 to 12.1%. These results align with those reported by Michelsen et al. [16], who documented a median STP of 17.05 g/L and a mean %Brix value of 8.5%. Similarly, Deelen et al. [10] reported a Mean $\pm$ SD STP of 6 $\pm$ 0.8 g/dL, ranging from 4.4 to 8.8 g/dL, and a Mean $\pm$ SD %Brix of 9.2 $\pm$ 0.9%, spanning from 7.3 to 12.4%.

In this study, most calves (58%) exhibited an STP level greater than 10 g/L, indicating sufficient transfer of passive immunity. Calves are classified as having poor passive immunity when their STP concentration is <10 g/L, measured between 1 and 7 days of age [12]. Our results indicated that 42% of samples were classified as «poor TPI», 19% were classified as «fair TPI» and 39% were classified as «excellent TPI».

These data were classified according to the criteria established by Lombard et al. [8] in the United States, who categorized passive immunity levels into three groups based on calf serum Brix values: "poor" (<8.4%), "fair" (8.4–9.4%), and "excellent" (>9.4%). This classification aligns with previous studies defining successful transfer of passive immunity (TPI) as serum Brix values greater than 9.4%, and TPI failure as values below 8.4%. [15]. Our results were higher than those reported by an experiment conducted in the US Department of Agriculture [17], who found that 35.5% of samples had a good passive immunity  $\geq$ 25 g/L and 12% had poor passive immunity  $\leq$ 10 g/L. Fernandez-Novoa et al. [18] reported that 35.3% of calves achieved the highest category of PIT. Their findings indicated that calves receiving greater volumes of colostrum were more likely to fall into superior transfer of passive immunity (TPI) categories. Calves with STP concentrations >15 g/L are less likely to develop respiratory disease [19]. Another study carried out in Canada on Holstein calves by Deelen et al. [10], percent Brix values <8.4 correlate

with failure or poor passive immunity, paralleling the <10 g/L serum IgG threshold.

Michelsen et al. [16] founded the distribution of calves across four serum total protein categories «poor, fair, good, and excellent» as 29%, 23.5%, 22.3%, and 25.2%, respectively. Correspondingly, the distribution based on %Brix values was 35.1%, 30.9%, 18.5%, and 15.5%, respectively, reflecting variability in PIT efficacy.

In Germany, Sutter et al. [20], delineated the distribution of passive immunity status among calves categorized by transfer of passive immunity (TPI) levels as follows: 4.8% classified as poor, 29.5% as fair, 28.3% as good, and 37.4% as excellent TPI. The findings reveal that the main contributor to unsuccessful PIT is the low quality of colostrum, with 36% of calves that failed to acquire immunity having consumed colostrum containing less than 50 g/L of IgG. Additionally, the timing of the first feeding (within 4 hours following birth) plays a role, as 6% of calves still experienced failure despite receiving colostrum with high IgG levels, approximately 90 g/L.

Our results reveal that calf sex did not significantly influence STP concentration ( $P>0.05$ ); In contrast, Cavi-rani et al. [21] and Guerrero-Pincay et al. [22] reported higher STP levels in females than in males, with the latter finding a difference of 5.77 g/dL versus 5.01 g/dL.

STP concentrations varied according to calf age at sampling. On day 1, calves exhibited a Mean $\pm$ SD STP of 44.72 $\pm$ 5.5 g/L. This value decreased to 23.8 $\pm$ 9.24 g/L on day 2 and reached 18.45 $\pm$ 7.7 g/L by day 3. A gradual decline was observed after day 4, with Mean $\pm$ SD STP decreasing to 3.51 $\pm$ 0.92 by day 5. These findings are consistent with previous reports showing that STP concentrations peak shortly after colostrum ingestion and subsequently decline during the first days of life as ma-

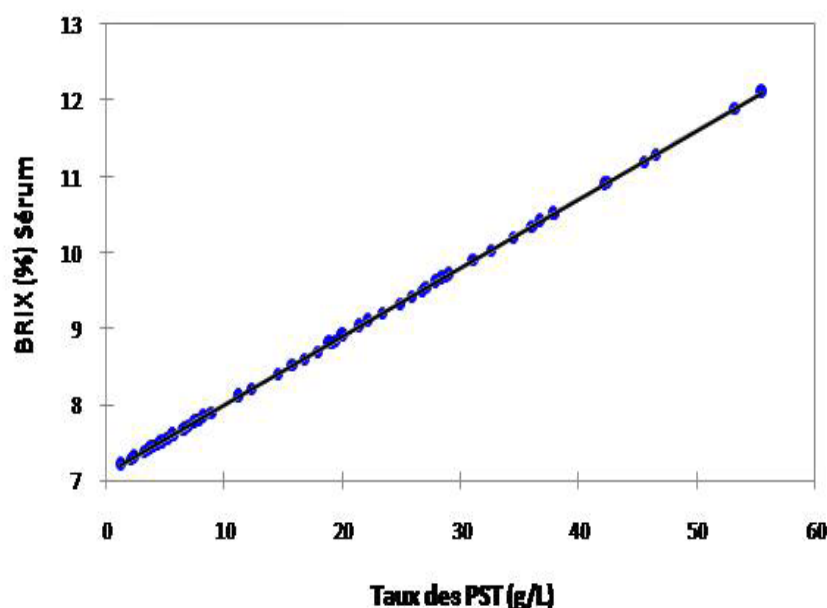


**Figure 2.** Percentage Brix, compared with STP of calf serum

ternal immunoglobulins are metabolized and redistributed [8].

This study revealed that calving type had a significant effect on PIT ( $P < 0.05$ ). Specifically, unassisted calving (score 1) differed significantly from both calving with easy assistance (score 2) and calving requiring mechanical assistance (score 3), whereas no significant difference was observed between scores 2 and 3. We found that total serum protein concentrations decreased progressively with increasing calving difficulty, averaging 27.5 g/L (9.6% Brix) following unassisted calving (score 1), declining to 5.6 g/L (7.6% Brix) with easy help (score 2), and reaching 2.88 g/L (7.35% Brix) in cases requiring mechanical assistance (score 3). According to Sutter et al. [20] calves that required assistance during calving exhibited significantly lower STP concentrations as measured by %Brix ( $P < 0.01$ ). Dystocia had a significant negative impact on STP levels, with calves needing calving assistance showing reduced %Brix values (score 2:  $-0.08 \pm 0.03\%$  Brix,  $P = 0.01$ ; score 3:  $-0.14 \pm 0.03\%$  Brix,  $P < 0.01$ ) compared to those born without assistance. No statistically significant difference was observed between calves assisted by one versus two persons ( $P = 0.14$ ). Additionally, Fernandez-Novo et al. [18] reported that dystocic calving was associated with a 0.492-fold decrease in colostrum quality relative to normal calving. As a result, the weakened newborn calf tends to be slower to stand and nurse, consuming less colostrum, which impairs the transfer of immunity.

#### 4.1. Correlation

The current study reveals a strong positive correlation between Brix refractometer reading and total serum protein concentration ( $r = 1$ ). Our results are similar to those of Deleen et al. [10], but slightly higher than those found by Giammarco et al. [23] who reported correlation of  $r = 0.98$ . Notably, Morrill et al. [13] did not compare Brix results with STP measurements on the same samples.

#### 4.2. Test characteristic

In the present study, the sensitivity and specificity of %Brix at a threshold of 7.8% were 100% and 69%, respectively. These findings align closely with those reported by Morrill et al. [13], who documented a sensitivity of 98.9% and specificity of 38.9%, at the same threshold. A value of  $< 8.4\%$  Brix most accurately predicted FPT, providing a reasonable estimate of serum total protein concentration in the majority of calves tested. Our results at the 8.4% Brix threshold demonstrated a sensitivity of 89% and specificity of 85%, marginally higher than the values reported in France by Mugnier et al. [24], who observed a sensitivity and specificity of 86.5% and 83.8%, respectively, at the same cutoff. Comparatively, Wenz et al. [25], reported sensitivity and specificity values of 93.7 and 77.8%, respectively, at an 8.3% Brix cutoff. Furthermore, Lombard et al. [8] emphasized that there was no significant difference in mortality rates between calves with serum total protein concentrations below 10 g/L and those with concentrations ranging from 10.0 to 17.9 g/L, highlighting consideration regarding current

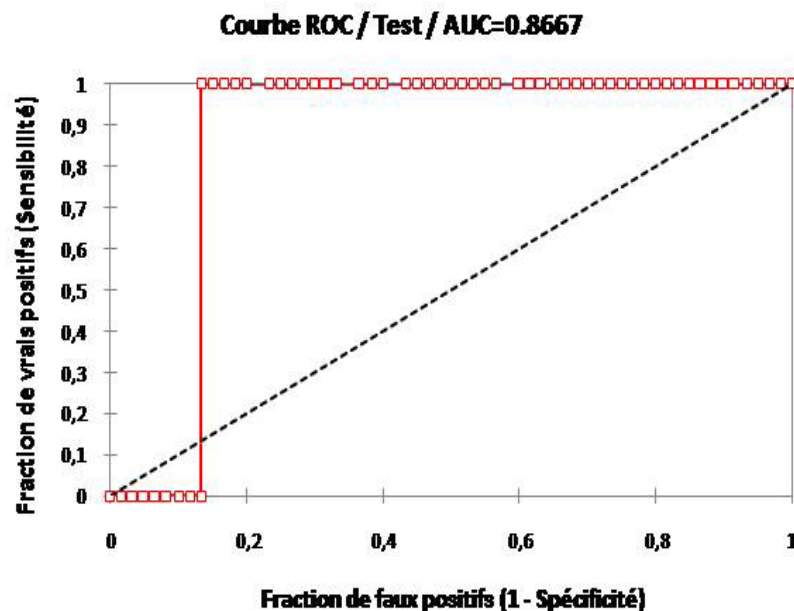


**Figure 3.** ROC curve of the true positive rate against the false positive rate for the different percentage Brix

failure of passive transfer immunity (FPT) cutoff points. The optimal threshold according to the Youden index is therefore 8.4% with an index of 0.74. Almost the same Youden index (0.7) was reported by Mugnier et al. [24].

ROC: The area under the curve (AUC) found by current study was 0.86; this result was very close (0.88) to that reported by Mugnier et al. [24].

This ROC curve demonstrates that the digital refractometer is a very effective and reliable instrument for assessing the success of PIT in calves. Its ability to achieve high sensitivity with a low false positive rate, confirmed by a solid AUC of 0.8667, makes it a valuable tool for farmers and veterinarians to make informed decisions regarding calf health.

## 5. Conclusion

These findings highlight that transfer of passive immunity is a key factor influencing the survival of neonatal calves. In herd management, the use of a digital refrac-

tometer offers a practical and cost-effective method for on-site assessment of immunoglobulin transfer. This approach can help identify calves at risk of FPT; a condition linked to higher neonatal morbidity, mortality, and impaired growth, particularly in indigenous cattle under extensive pasture conditions.

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## Compliance with ethical guidelines

Experimental procedures approved by the Institutional Committee for the Protection of Animals of the National

**Table 2.** Diagnostic characteristics of Brix percentage for assessing failure of passive immunity transfer

| Test Characteristic (%) |             |             |                  |
|-------------------------|-------------|-------------|------------------|
| Cutoff Point            | Sensitivity | Specificity | Youden Index (J) |
| 7.8%                    | 100         | 69          | 0.69             |
| 8.4%                    | 89          | 85          | 0.74             |
| 9%                      | 72          | 92          | 0.64             |

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We hereby declare all ethical standards have been respected in preparation of the submitted article.

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### Authors' contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interception of the results and drafting of the manuscript. Each author approved the final version of the manuscript for submission.

### Conflict of interest

The authors declared that no conflict of interest.

### Data availability

All data of this study are available on request from the corresponding author.

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