

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.

Objective: The objective of the current study was to determine the prevalence of sufficient passive immunity transfer in order to assess neonatal survival capacity under natural environmental conditions.

Material and 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 total serum proteins levels using a digital refractometer.

Results: The mean of serum total protein concentration in calves was 18.56 g/L (SD \pm 15.00), and the mean serum Brix percentage was 8.8 % (SD \pm 1.3). Total serum protein correlated strongly with % Brix ($r = 1.00$). 58% of calves presented a serum total protein 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 passive immunity transfer was influenced by the calving score (P value = 0.03). However, the sex of the calf had no impact on the success of this transfer (P value = 0.07). Test characteristics were evaluated to assess the failure of passive transfer determined as serum total protein 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 total serum protein in calves to facilitate monitoring the success of passive transfer to calves born in mountainous regions with free-range grazing.

Key words: Bovine- Brix- Colostrum- Refractometer.

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

42 rich in immunoglobulins, nutrients and bioactive factors that provide immunological
43 protection and nutritional support to bovine neonates [3,4]. Calves are born almost
44 agammaglobulinemic with a complete but immature immune system. They acquire passive
45 immunity after birth by absorbing immune-related cells and proteins, primarily IgG, from
46 colostrum through the gut.

47 Local Algerian cows are well adapted to their natural environment, which facilitates
48 calving. Their slightly inclined pelvis and wider pelvic opening ease the calf's passage

49 during birth, significantly reducing the risk of obstetrical complications. Thanks to these
50 traits, the cows calve naturally without human intervention, ideal for a free-range grazing
51 system. After birth, they effectively nurse their calves, ensuring a good start for the newborn.
52 This autonomy supports a more rustic husbandry style, less dependent on constant farmer
53 supervision [5].

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

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

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

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

69 Indirect tests, such as Brix refractometry [11], offer a cost-effective, on-site alternative for
70 measuring serum total protein (STP) concentration and estimating IgG in colostrum. STP
71 evaluation via refractometry is inexpensive, easy to use, and suitable for field application
72 [12]. Brix degrees correlate highly ($r = 0.87$ to 0.93) with serum IgG concentration [10, 13].
73 Calves with IgG levels above 10 g/L are considered protected; FPT presence correlates with
74 health and performance issues [8]. Thus, STP evaluation can be used on farms to monitor
75 colostrum management and predict calf health.

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

79

80 2- Materiel and methods:

81 2-1- Assessment of passive immunity transfer

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

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

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

105 **2-2 The statistical processing** of data was generated with Excel Stat 2014.

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

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

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

121 **3- Results:**

122 To the best of our knowledge, this is the first study conducted to estimate the passive
123 immunity transfer in the indigenous cattle population in Algeria.

124 In this study, the mean of STP concentration was 18.56 g/L (SD ± 15.00; range 1.2–55.5
125 g/L), and the average Brix percentage was 8.8% (SD ± 1.3; range 7.2–12.1%). Descriptive
126 statistics for the serum samples are shown in Table 1.

127
128 **Table 1:** Descriptive statistics of calf serum sample measurements

	Number (%)	Mean		SD		Minimum		Maximum		
		STP(g/L)	Brix%	STP(g/L)	Brix%	STP(g/L)	Brix%	STP(g/L)	Brix%	
Total	62	18.56	8.8	15.00	1.3	1.2	7.2	55.5	12.1	
Sex of calf										
Male	38 (61%)	17.98	8.7	12.79	1.15	1.2	7.2	55.5	12.1	
Female	24 (39%)	20.11	8.9	10.12	0.91	4.3	7.5	42.1	10.9	
Type of calving										
¹Score 1	37 (60%)	27.5	9.6	9.68	0.87	5.18	7.6	55.5	12.1	
²Score 2	20 (32%)	5.6	7.6	1.51	0.13	3.41	7.4	11.2	8.1	
³Score 3	5 (8%)	2.88	7.35	0.99	0.09	1.2	7.2	4.52	7.5	
Age of calf (day)										
1	8	44.72	11.13	5.5	0.5	34.4	10.2	55.5	12.1	
2	15	23.8	9.24	6.9	0.6	11.2	8.1	36.6	10.4	
3	18	18.45	8.76	7.7	0.7	5.2	7.56	37.7	10.5	
4	12	5.87	7.62	1.23	0.11	3.41	7.4	8.18	7.83	
5	9	3.51	7.4	0.92	0.08	1.2	7.2	4.74	7.52	

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

131 The calves were classified by sex, with 38 males (61%) and 24 females (39%). In male
132 calves, the mean serum total protein (STP) concentration was 17.98 g/L (SD ± 12.79; range:
133 1.2–55.5 g/L), and the mean %Brix value was 8.7% (SD ± 1.15; range: 7.2–12.1%). In
134 female calves, the mean STP was 20.11 g/L (SD ± 10.12; range: 4.3–42.1 g/L), and the mean
135 %Brix was 8.9% (SD ± 0.91; range: 7.5–10.9%).

136 Based on calving conditions, 60% of calves were born without assistance (Score 1), 32%
137 required mild assistance (Score 2), and 8% required mechanical assistance (Score 3). One
138 case of neonatal mortality following cesarean delivery was recorded and excluded from the
139 analysis. The mean STP concentrations for calves with Scores 1, 2, and 3 were 27.5 g/L (SD
140 ± 9.68), 5.6 g/L (SD ± 1.51), and 2.88 g/L (SD ± 0.99), respectively.

141 The evolution of serum total protein (STP) concentrations according to calf age (in days)
142 indicate that on day 1 (n = 8), calves exhibited the highest mean STP value (44.72 g/L ± 5.5),
143 indicating effective passive transfer of immunoglobulins shortly after colostrum intake. A
144 marked decline was observed on day 2 (n = 15), with mean STP decreasing to 23.8 g/L (±
145 6.9), followed by a further reduction on day 3 (n = 18; 18.45 g/L ± 7.7). From day 4 onward,
146 STP concentrations decreased sharply, reaching 5.87 g/L (± 1.23) on day 4 (n = 12) and 3.51
147 g/L (± 0.92) on day 5 (n = 9). This progressive decline indicates a rapid reduction in
148 circulating immunoglobulins and total protein after the initial colostrum absorption period.

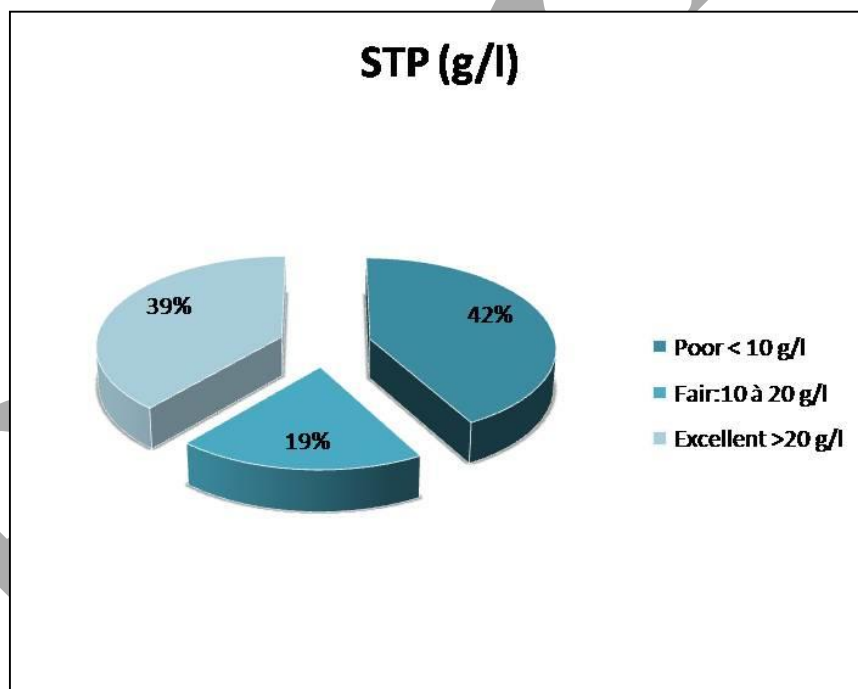
149 Precise information regarding the timing of first colostrum ingestion was unavailable. This
150 was mainly due to the extensive husbandry system in mountainous areas, where calves are
151 born on pasture and remain with their dams, preventing direct observation and accurate
152 recording of colostrum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

156 Analysis of variance (ANOVA) of our data indicated that the type of calving influenced
157 blood levels of STP, and consequently the success of passive immunity transfer in the calf (P
158 value <0.05). Highly significant differences were observed between calving scores 1 and 2,
159 and between scores 1 and 3, with 95% confidence intervals of [17.28–25.50] and [18.10–
160 30.98], respectively. In contrast, no statistically significant difference was found between
161 scores 2 and 3 (95% CI: -0.92 to 7.22).

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

164 58 % presented a sufficient transfer of passive immunity with STP concentrations higher than
165 10 g/L. Among these calves, 19% were classified as «fair TPI» with a total serum proteins
166 level between 10 and 20 g/L corresponding to %Brix 8.4 - 9.4%, while 39% have a level
167 higher than 20 g/L with Brix>9.4% (classified as «excellent TPI»).

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169

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Figure 1: Percent of passive immunity transfer

171 The percentage of male calves with a STP concentration higher and lower than 10 g/L was
172 53% and 47% respectively. About females, this percentage was 68% and 32% respectively.
173 We determined that the sex of the calf had no significant effect on the total serum protein
174 level (P value = 0.07) with 95 % CI [-5,92 ; 11,69].

175

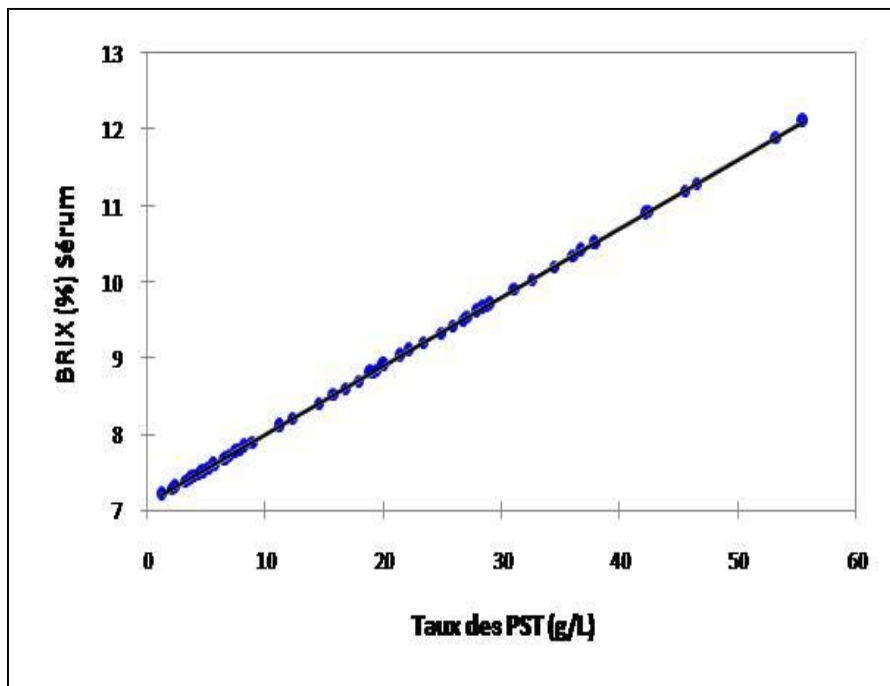
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179 **3-1- Correlation:**

180 Correlation analysis showed that total serum protein (STP) levels were strongly positively
 181 correlated with %Brix ($r = 1.00$) (Figure2).



182

183 **Figure 2:** Percentage Brix %, compared with serum total protein (STP) of calf serum

183

184 **3-2- Test Characteristics**

185

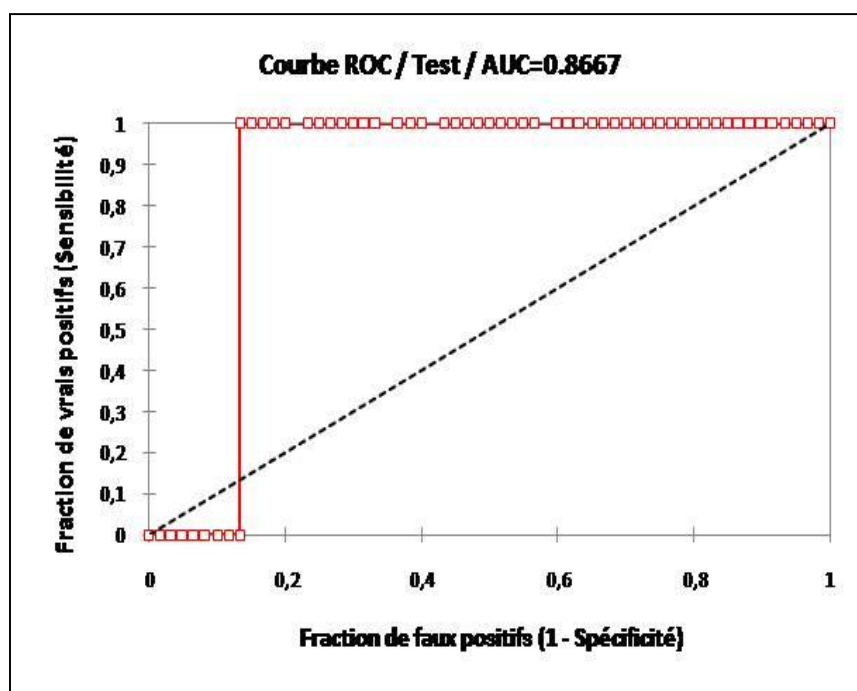
186 The absence of a gold standard method, such as radial immunodiffusion, limits the
 187 interpretation of the calculated sensitivity and specificity. Therefore, these estimates should
 188 be interpreted with caution.

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

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

196 **Table 2:** Test characteristics for and percentage Brix %

Cutoff point	Test characteristic (%)		Youden index (J)
	Sensitivity (%)	Specificity (%)	
7,8 %	100	69	0,69
8,4 %	89	85	0,74
9 %	72	92	0,64



198

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

201 4- Discussion:

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

209 In the current study, the mean STP was 18.56 g/L (SD \pm 15.00) with a range from 1.2 to 55.5
210 g/L, and the mean %Brix was 8.8% (SD \pm 1.3) with a range from 7.2 to 12.1%. These results
211 align with those reported by Michelsen et al. [16], who documented a median STP of 17.05
212 g/L and a mean % Brix value of 8.5%. Similarly, Deelen et al. [10] reported a mean STP of
213 6.0 g/dL (\pm 0.8 SD), ranging from 4.4 to 8.8 g/dL, and a mean %Brix of 9.2% (\pm 0.9 SD),
214 spanning from 7.3 to 12.4%.

215 In this study, most calves (58%) exhibited an STP level greater than 10 g/L, indicating
216 sufficient transfer of passive immunity. Calves are classified as having poor passive
217 immunity when their serum total protein concentration is $<$ 10 g/L, measured between 1 and 7
218 days of age [12]. Our results indicated that 42% of samples were classified as «poor TPI»,
219 19% were classified as «fair TPI» and 39% were classified as «excellent TPI».

220 These data were classified according to the criteria established by Lombard et al. [8] in the
221 United States, who categorized passive immunity levels into three groups based on calf serum
222 Brix values: "poor" ($<$ 8.4%), "fair" (8.4–9.4%), and "excellent" ($>$ 9.4%). This classification
223 aligns with previous studies defining successful transfer of passive immunity (TPI) as serum
224 Brix values greater than 9.4%, and TPI failure as values below 8.4%. [15]. Our results were

225 higher than those reported by an experiment conducted in the US Department of Agriculture
226 [17], who found that 35.5% of samples had a good passive immunity ≥ 25 g/L and 12% had
227 poor passive immunity ≤ 10 g/L. Fernandez-Novio et al.[18] Reported that 35.3% of calves
228 achieved the highest category of passive immunity transfer. Their findings indicated that
229 calves receiving greater volumes of colostrum were more likely to fall into superior transfer
230 of passive immunity (TPI) categories. Calves with STP concentrations >15 g/L are less likely
231 to develop respiratory disease [19]. Another study carried out in Canada on
232 Holstein calves by Deelen et al.[10], Percent Brix values <8.4 correlate with failure or poor
233 passive immunity, paralleling the <10 g/L serum IgG threshold.

234 Michelsen et al.[16] Founded the distribution of calves across four serum total protein
235 categories «poor, fair, good, and excellent» as 29.0%, 23.5%, 22.3%, and 25.2%,
236 respectively. Correspondingly, the distribution based on %Brix values was 35.1%, 30.9%,
237 18.5%, and 15.5%, respectively, reflecting variability in passive immunity transfer efficacy.

238 In Germany, Sutter et al.[22], delineated the distribution of passive immunity status among
239 calves categorized by transfer of passive immunity (TPI) levels as follows: 4.8% classified as
240 poor, 29.5% as fair, 28.3% as good, and 37.4% as excellent TPI. The findings reveal that the
241 main contributor to unsuccessful passive immunity transfer is the low quality of colostrum,
242 with 36% of calves that failed to acquire immunity having consumed colostrum containing
243 less than 50 g/l of IgG. Additionally, the timing of the first feeding (within 4 hours following
244 birth) plays a role, as 6% of calves still experienced failure despite receiving colostrum with
245 high IgG levels, approximately 90 g/l.

246 Our results reveal that calf sex did not significantly influence STP concentration (P value $>$
247 0.05); In contrast, Cavirani et al. [20] and Guerrero-Pincay et al. [21] reported higher STP
248 levels in females than in males, with the latter finding a difference of 5.77 g/dL versus 5.01
249 g/dL.

250 Serum total protein (STP) concentrations varied according to calf age at sampling. On day 1,
251 calves exhibited a mean STP of 44.72 g/L (SD \pm 5.5). This value decreased to 23.8 g/L (SD \pm
252 9.24) on day 2 and reached 18.45 g/L (SD \pm 7.7) by day 3. A gradual decline was observed
253 after day 4, with mean STP decreasing to 3.51 g/L (SD \pm 0.92) by day 5. These findings are
254 consistent with previous reports showing that STP concentrations peak shortly after
255 colostrum ingestion and subsequently decline during the first days of life as maternal
256 immunoglobulins are metabolized and redistributed [8].

257 This study revealed that calving type had a significant effect on passive immunity transfer (P
258 $<$ 0.05). Specifically, unassisted calving (score 1) differed significantly from both calving
259 with easy assistance (score 2) and calving requiring mechanical assistance (score 3), whereas
260 no significant difference was observed between scores 2 and 3. We found that total serum
261 protein concentrations decreased progressively with increasing calving difficulty, averaging
262 27.5 g/L (9.6% Brix) following unassisted calving (score 1), declining to 5.6 g/L (7.6% Brix)
263 with easy help (score 2), and reaching 2.88 g/L (7.35% Brix) in cases requiring mechanical
264 assistance (score 3). According to Sutter et al.[22] Calves that required assistance during
265 calving exhibited significantly lower serum total protein (STP) concentrations as measured
266 by % Brix (P $<$ 0.01). Dystocia had a significant negative impact on STP levels, with calves
267 needing calving assistance showing reduced % Brix values (score 2: $-0.08 \pm 0.03\%$ Brix, P =

268 0.01; score 3: $-0.14 \pm 0.03\%$ Brix, $P < 0.01$) compared to those born without assistance. No
269 statistically significant difference was observed between calves assisted by one versus two
270 persons ($P = 0.14$). Additionally, Fernandez-Novo et al, [18] reported that dystocic calving
271 was associated with a 0.492-fold decrease in colostrum quality relative to normal calving. As
272 a result, the weakened newborn calf tends to be slower to stand and nurse, consuming less
273 colostrum, which impairs the transfer of immunity.

274 **4-1- Correlation:**

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

280 **4-2- Test characteristic:**

281 In the present study, the sensitivity and specificity of % Brix at a threshold of 7.8% were
282 100% and 69%, respectively. These findings align closely with those reported by Morrill et
283 al.[13], who documented a sensitivity of 98.9% and specificity of 38.9%, % at the same
284 threshold. A value of $<8.4\%$ Brix most accurately predicted FPT, providing a reasonable
285 estimate of serum total protein concentration in the majority of calves tested. Our results at
286 the 8.4% Brix threshold demonstrated a sensitivity of 89% and specificity of 85%, marginally
287 higher than the values reported in France by Mugnieret al.[24], who observed a sensitivity
288 and specificity of 86.5% and 83.8%, respectively, at the same cutoff. Comparatively, Wenz et
289 al.[25], reported sensitivity and specificity values of 93.7 and 77.8%, respectively, at an 8.3%
290 Brix cutoff. Furthermore, Lombard et al.[8]emphasized that there was no significant
291 difference in mortality rates between calves with serum total protein concentrations below
292 10 g/l and those with concentrations ranging from 10.0 to 17.9 g/L, highlighting
293 consideration regarding current failure of passive transfer immunity (FPT) cutoff points. The
294 optimal threshold according to the Youden index is therefore 8.4% with an index of 0.74.
295 Almost the same Youden index (0.7) was reported by Mugnieret al. [24].

296 ROC (Receiver Operating Characteristic): The Area under the curve (AUC) found by current
297 study was 0.86; this result was very close (0.88) to that reported by Mugnieret al.[24].

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

303

304 **5- Conclusion**

305 These findings highlight that transfer of passive immunity is a key factor influencing the
306 survival of neonatal calves. In herd management, the use of a digital refractometer offers a
307 practical and cost-effective method for on-site assessment of immunoglobulin transfer. This
308 approach can help identify calves at risk of failure of passive transfer; a condition linked to
309 higher neonatal morbidity, mortality, and impaired growth, particularly in indigenous cattle
310 under extensive pasture conditions.

311

312 **Acknowledgments**

313 The authors wish to express their gratitude to the veterinarians and livestock farmers of the
314 mountainous regions of Guelma for their contribution to the collection of newborn calf serum
315 used in this study. They also thank the director and staff of the Animal Production,
316 Biotechnology and Health Laboratory at Mohamed-Cherif Msaadia University of Souk-Ahras
317 for their technical assistance throughout this project.

318 **Ethics:** We hereby declare all ethical standards have been respected in preparation of the
319 submitted article.

320 **Ethical approval**

321 Experimental procedures approved by the Institutional Committee for the Protection of
322 Animals of the National Administration of Higher Education and Scientific Research of
323 Algeria (98-11, Act of 22 August 1998).

324

325 **Data availability**

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

327

328 **Conflict of Interest:** The authors declare that they have no conflict of interest.

329 **Funding**

330 No finding.

331

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