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Effect of Birth Season, Sex and Birth Weight on Total Serum Protein Levels in Holstein-Friesian Calves in Northern Mexico

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ABSTRACT

Background: Timely colostrum intake improves passive immunity transfer in calves, enabling proper immune system development and positively impacting subsequent productive and reproductive performance. The objective of the present study was to determine whether the birth season (BS), sex and birth weight (BW) affect total serum protein levels (TSPL) in Holstein-Friesian calves.

Methods: A total of 4464 Holstein-Friesian calves were evaluated. All the calves were weighed at birth and fed with 4 L of high-quality colostrum (≥27 °Bx) within the first 2 hours of life. Blood samples were collected between 24 and 48 hours after birth to determine TSPL. One-way and two-way analyses of variance were performed, along with pairwise mean comparisons, using Tukey's honestly significant difference post hoc test. Differences were statistically significant (P≤0.05). Additionally, point-biserial and pearson correlation coefficients were used to conduct a correlation analysis among the variables, both with a 95% confidence level.

Result: Out of all the calves, 57.82% were female and 42.18% were male. Males had a greater birth weight (BW) (41.28±6.05 kg) than females (37.61±5.14 kg) (P≤0.05). The highest number of births took place in winter (1440), followed by summer, autumn and spring; however, the greatest average BW was recorded in spring: 39.88±5.82 kg. The mean TSPL was 5.99±0.80 g dL^{-1}. Based on BS, sex and BW, differences (P≤0.05) in TSPL and in the interactions between these variables were established.

Key words: Dairy calves, Hot climate, Passive immunity.

INTRODUCTION

Heat stress (HS) has a major impact on the dairy industry, because it reduces production, affects immune responses and reduces reproductive performance (Collier *et al*., 2006; Hansen, 2009; Lendez *et al*., 2021). HS during late gestation lowers total protein concentration in bovine colostrum (Dahl *et al*., 2020), jeopardizing the passive immunity transfer (PIT) to calves born during periods of heat stress (Almoosavi *et al.*, 2020). Therefore, HS is a major issue, given that calves rely on the protection of the PIT until their own immune systems are appropriately developed (Godden *et al*., 2019; Lopez and Heinrichs, 2022; Weaver *et al*., 2000). Consequently, timely colostrum intake during the first few hours of life enhances the PIT (Ferreira-Alcântara *et al*., 2026; Male-Here *et al*., 2026; Yang *et al*., 2025). Raboisson *et al.* (2016) estimated that-from an economic point of view-failures in PIT costed 60 euros per dairy calf.

A simple and widely accepted method to assess PIT involves measuring total serum protein levels (TSPL), which can be easily performed using a refractometer between 24 and 48 hours after birth (Dunn *et al*., 2017). The primary variables that influence TSPL are the speed, quality and quantity of colostrum consumed by a calf (Godden, 2008; Stancheva and Penev, 2025). Birth season (BS), sex and birth weight (BW) also influence serum protein levels in calves (Immler *et al*., 2022; Santos *et al*., 2024; Trzebiatowski *et al*., 2025; Weaver *et al*., 2000). Likewise, individual factors of the calf-including sex and BW- influence TSPL (Aghakhani *et al*., 2023; Brereton *et al*., 2024; Cortes-Guerra *et al*., 2024; Song *et al*., 2026).

PIT is essential for the health and survival of calves in the first weeks of life, taking into account that a failure in the aforementioned transfer has been associated with an

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increase of morbidity, mortality and a reduced growth rate in calves (Lora *et al*., 2018; Peña-Revuelta *et al*., 2024). A successful PIT is important for milk producers for numerous reasons, including effects on long-term calf productivity, lower milk production during first and second lactations and a higher culling rate during the first lactation week (Hayes *et al*., 2019, 2021). In that sense, several investigations have demonstrated that high PIT levels are associated with better health outcomes and productivity at different stages in calves (Crannell and Abuelo, 2023; Lombard *et al*., 2020; Sutter *et al*., 2023). This is particularly important for dairy heifers, as their development and health before weaning influence in a significant way their milk production potential and their permanence in the dairy herd (Abuelo *et al*., 2021; Brickell *et al*., 2009; Hayes *et al*., 2019, 2021).

Despite this situation, global research involving large numbers of newborns and the correlation between TSPL in dairy calves and variables such as BS, sex and BW is very limited. This gap is even larger in Mexico's primary dairy-producing region (*i.e.*, the comarca lagunera), where the relationship between TSPL in calves-along with factors such as BS, sex and BW-has not been documented yet. Consequently, the hypothesis and objective of this study were to determine whether BS, sex and BW impact TSPL of Holstein-Friesian calves in the comarca lagunera, Mexico (CLM). Thus, the objective of the present study was to determine if SB, sex and BW affect TSPL in Holstein-Friesian calves. To improve the productive success of the bovine dairy production units and increase the income of livestock farming families, with a holistic vision focused on achieving true sustainability in the CLM (Armijo-Nájera *et al*., 2026; Navarrete-Molina *et al*., 2019).

MATERIALS AND METHODS

Description of the study location

All procedures and animal handling involved in this study complied with national (NAM, 2002) and international (FASS, 2010) guidelines regarding animal ethics, care and welfare. The study was conducted from May 1, 2024, to April 30, 2026. It took place at a highly mechanized dairy production facility (2500 lactating cows) located in the southwestern part of the state of coahuila, Mexico (25°N), in the region known as the comarca lagunera. This region is located in north-central Mexico (25°4′N, 103°2′O, 1113 masl) and features a semi-arid ecosystem with a mean annual temperature of 23.8°C, maximum temperatures of 41.0°C in summer and minimum temperatures of -1.0°C in winter and a mean annual precipitation of 230 mm. The relative humidity of the region ranges from 12% to 61% and the photoperiod varies from a maximum of 13 h 41 min during the summer solstice (June) to a minimum of 10 h 19 min during the winter solstice (December) (CONAGUA, 2025; INEGI, 2010).

Study animals, housing and colostrum management

The study population consisted of 4464 Holstein-Friesian calves. The calves were separated from their dams at birth; immediately afterwards, their navels were disinfected with a 7% iodine solution (Tintura de yodo, NUPLEN Comercializadora, Torreon, México) and they were weighed using a 0.1 kg precision portable scale (Modelo ERFP SBP, Basculas Revuelta, Torreon, México). Subsequently, they were housed in individual wooden pens (2 × 1.5 m) with metal roofs and sand bedding, located in a well-aired area within the dairy facility. All calves were fed using a bottle with a teat and received 4 L of pasteurized colostrum (62°C for one hour) within the first 2 hours after birth, followed by a second feeding of 2 L, eight hours after the initial feeding. All calves were fed a total of 6 L of colostrum within 10 hours of birth. The colostrum came from the existing stock of the dairy farm. Only high-quality colostrum can be part of this bank (≥a 27 °Bx) (Flynn *et al*., 2025).

At the dairy farm, colostrum collection and storage take place within the first 2 hours postpartum, using the following procedure: the hind legs of the cows are restrained, then the udder is washed with clean water and dried with disposable paper towels. Next, all available colostrum is collected from the four teats by hand-milking into a clean and sterilized container. The colostrum is then thoroughly mixed (homogenized) and its quality is assessed using a °Bx refractometer (MISCO Palm Abbe #PA203 US Patent 10880). If the colostrum records < 27°Bx, it fails to meet the minimum quality standard and, consequently, it is discarded. Otherwise, the colostrum is poured into clean plastic bottles labeled with the ID of the cow, the collection date and the °Bx value. Finally, the colostrum is frozen at -24°C in a Rhino freezer (model Congeco-16, Rhino Maquinaria, Lomas de Atizapan, Mexico) until its use.

Measurement of the variables

As previously stated, calf BW was recorded immediately after delivery. Calf sex was determined by physical observation of its reproductive organs. BS categories were defined as follows: spring (March 22-June 21); summer (-June 22- September 21); autumn (September 22-December 21) and, finally, winter (December 22-March 21).

Blood samples were collected from all calves between 24 and 48 hours after birth, in order to determine TSPL. Sampling involved obtaining 5 mL of blood *via* jugular venipuncture using 9 mL BD Vacutainer® tubes (Franklin Lakes, New Jersey, USA), containing a clot activator. Blood samples were refrigerated 24 hours prior to serum separation. The samples were then centrifuged at 3500 revolutions per minute for 15 minutes at 4°C in a VELAB VE-4000 digital centrifuge (VELAB, Tlalpan, Mexico). Subsequently, TSPL were determined, using a drop of serum placed on a digital refractometer for dairy operations (MISCO Digital-Dairy™, Solon, OH, USA). Before applying the serum drop, the device was calibrated with a drop of distilled water and the reading window of the refractometer was cleaned with a clean piece of paper. Following the reading, the refractometer was recalibrated with a drop of distilled water. All measurements were performed at room temperature (approximately 30°C).

Statistical analysis of data

The shapiro-wilk test was used to evaluate the normality of each response variable. Additionally, Levene's test was used to verify the equality of variances. Subsequently, one-way and two-way analyses of variance (ANOVA) were performed, complemented by Tukey's honestly significant difference post hoc test. The results included the mean and standard error of the mean for the response variables. Differences were considered statistically significant if Pd≤0.05. Additionally, a correlation analysis was conducted among the studied variables using the point-biserial correlation coefficient (r_{pb}) with a 95% confidence level for the correlations between the sex variable (dichotomous) and the remaining variables (non-dichotomous). Furthermore, pearson's correlation coefficient (r) was used in the correlation analysis between the numerical variables (*i.e.*, BW, birth date in Julian days and TSPL). Both correlation analyses had a 95 % confidence level. All statistical analyses were performed using the Minitab® version 20.44 software (Minitab, 2021).

RESULTS AND DISCUSSION

Table 1 shows the results obtained for BS and sex variables interaction and their influence on the BW of Holstein-Friesian calves in northern Mexico. Out of all the records analyzed (n = 4464), 57.82 % were from females and the remaining 42.18% were from males. Regarding sex, males recorded a greater BW average (41.28±6.05 kg) than females (37.61±5.14 kg). Regarding the BS, winter recorded the highest number of births, followed by summer, autumn and spring, with 1440, 1137, 1110 and 777 births, respectively. However, spring was the season that recorded the greatest BW average (39.88±5.82 kg). The ANOVA revealed differences (P≤0.05) in BW based on BS, sex and the interaction between these two variables (Table 1).

Higher values were reported in spring, regarding the effect of BS on BW (Table 1). These results match the findings of Brost and Drackley (2025), who recorded the highest weights during summer. Another study reported that female calves born during warmer months had a higher milk production during their first lactation (Van Eetvelde *et al*., 2017). The results showed that male calves had a greater BW than females (P≤0.05) (Table 1). These results match the findings for dairy cows reported by Holland and Odde (1992) and by Meyer *et al.* (2001), who attributed the difference to fetal physiological differentiation, caused by androgenic hormone production and fetal metabolism. Furthermore, prenatal factors, sex and maternal conditions influence BW (Kamal *et al*., 2014) and neonatal performance (Westhoff *et al*., 2024).

Results for the total serum protein level variable showed a range of 7.2 g dL^{-1} for the 4464 calves analyzed, with a minimum recorded value of 2.5 g dL^{-1} and a maximum of 9.7 g dL^{-1}. The overall total serum protein level mean was 5.99 g dL^{-1}, with a standard deviation of 0.80 g dL^{-1}. Additionally, the results were classified according to the categories proposed by Godden *et al.* (2019) for TSPL. The results were as follows: 40.12 (1791), 24.01 (1072), 24.42 (1090) and 11.45% (511) recorded excellent (≥6.2 g dL^{-1}), good (5.8-6.1 g dL^{-1}), fair (5.1-5.7 g dL^{-1}) and poor (≤5.0 g dL^{-1}) levels, respectively. Table 2 includes the results of the interaction effect between BS and sex and their influence on the TSPL of Holstein-Friesian calves in northern Mexico. Spring recorded the highest TSPL, with a mean value of 6.16±0.94 g dL^{-1}, followed by autumn (6.08 ±0.72 g dL^{-1}), summer (5.97±0.82 g dL^{-1}) and winter (5.85±0.72 g dL^{-1}). Unlike the BW variable, females had a higher average of TSPL (6.03±0.79 g dL^{-1}) than males (5.93±0.80 g dL^{-1}). The ANOVA reported differences (P≤0.05) in TSPL, based on the BS, sex and the interaction between these two variables (Table 2).

The TSPL matched the findings of Weaver *et al.* (2000), who also pointed out that this variable is an indicator of PIT (Table 2). Colostrum provides not only immunoglobulins but also bioactive components essential for the immunological and metabolic development of the neonate (Silva *et al*., 2024). In this context, the results indicated adequate PIT in most animals when compared with the benchmarks proposed by Godden *et al.* (2019), regarding the relationship between serum immunoglobulin levels in calves and TSPL under proper colostrum management.

Table 1: Mean±mean's standard error of birth season x sex interaction in birth weight in Holstein-Friesian calves (n = 4464) in northern Mexico (25°N).

Season	Sex	N	Birth weight (kg)
Spring[a]	Female[b]	450	37.96±0.24[c]
	Male[a]	327	42.52±0.32[a]
Summer[b,c]	Female[b]	656	37.74±0.19[c]
	Male[a]	481	40.75±0.28[b]
Autumn[b]	Female[b]	606	37.94±0.21[c]
	Male[a]	504	41.20±0.29[b]
Winter[a]	Female[b]	869	37.09±0.18[d]
	Male[a]	571	41.10±0.23[b]
0.000*	0.000*		0.009*

[a-d] Values with different superscript lowercase letters in the same column represent differences (P≤0.05). * = P value.

The maternal environment during gestation, as well as seasonality and metabolic status, influence calf metabolism and immunity at birth (Immler *et al*., 2022; Jiang *et al*., 2026; Santos *et al*., 2024; Trzebiatowski *et al*., 2025).

The influence of BS and its effect on TSPL, recorded the highest levels in spring (Table 2). This result may be linked to variations in colostrum quality, maternal physiology and colostrum management practices (Cortes-Guerra *et al*., 2024). Furthermore, colostrum production and composition had seasonal variability (P≤0.05), influenced by factors such as HS, nutrition and the metabolic status of the cow (Westhoff *et al*., 2024). Additionally, research conducted under arid conditions has proven that HS reduces colostrum quality, directly impacting the PIT to the calf (Peña-Revuelta *et al*., 2024).

To improve the presentation of the data and results, the 4,464 records of the BW´s of the calves were classified into nine ranges: 16-20 kg (n = 14), 21-25 kg (n = 39), 26-30 kg (n = 220), 31 - 35 kg (n = 873), 36-40 kg (n= 1712), 41-45 kg (n = 1053), 46-50 kg (n = 433), 51-55 (n = 101) and, 56-60 (n = 19). However, to minimize errors in the results and their interpretation, ranges with a <100 sample size (n) were excluded from the analysis.

Table 2: Mean±mean's standard error of birth season x sex interaction in total serum protein levels (TSPL) in Holstein-Friesian calves (n = 4464) in northern Mexico (25°N).

Season	Sex	TSPL (g dL^{-1})
Spring^{a}	Female^{a}	6.21±0.04^{a}
	Male^{b}	6.09±0.05^{b}
Summer^{b}	Female^{a}	6.03±0.03^{b}
	Male^{b}	5.88±0.04^{c}
Autumn^{a}	Female^{a}	6.10±0.03^{b}
	Male^{b}	6.06±0.03^{b}
Winter^{c}	Female^{a}	5.90±0.02^{c}
	Male^{b}	5.78±0.03^{d}
0.000*	0.000*	0.015*

$^{a-d}$ Values with different superscript lowercase letters in the same column represent differences (P≤0.05). * = P value.

Accordingly, Table 3 includes the results of the interaction between BS and BW range (BWR) and their influence on the response variable of TSPL. The BWR that recorded the highest mean value for TSPL was 31-35 kg, followed by the 26-30 kg group with 6.06±0.81 and 6.03±0.80 g dL^{-1}, respectively. The ANOVA performed on TSPL and the interaction between BS and calf BWR reported significant differences (P≤0.05) for both factors and their interaction (Table 3). Likewise, differences (Pd≤0.05) were found in the interaction between sex and BWR and their influence on TSPL (Table 4).

Regarding the influence of BWR and BS on TSPL (Table 3), various researchers have correlated these levels with the efficiency of PIT in calves. These studies indicate that such transfer depends not only on the quantity of immunoglobulins ingested, but also on factors such as ingestion timing, intestinal absorption capacity and the biological quality of the colostrum (Flynn *et al*., 2025; Silva *et al*., 2024). Additionally, Virginio Júnior *et al.* (2026) have proven that the type and management of colostrum influence early intestinal microbial colonization and neonatal health. The results obtained from the comparison of the TSPL in females and males could be linked to individual calf factors, including biological variability and conditions at birth (Brereton *et al*., 2024; Dong-Hyeon *et al*., 2025).

The analyzed variables were subjected to a correlation analysis in order to complement the results (Fig 1). Highly significant correlations (P≤0.000) were found between TSPL and the sex and BW variables; however, the degree of correlation was low. A correlation was also conducted between sex and BW at the same significance level, showing a medium degree of correlation. A low but significant correlation (P≤0.05) was found between the birth date variable and the sex and BW variables (Fig 1). The low correlations documented may explain why the TSPL mainly depends on postnatal colostrum handling (Trzebiatowski *et al*., 2025) and the complexity of the factors that determine neonatal immunity (Cortes-Guerra *et al*., 2024). For this reason, newborns require adequate amounts of high-quality colostrum to avoid a poor PIT

Table 3: Mean±mean's standard error of the year's season x birth weight range in total serum protein levels (g dL^{-1}) in Holstein-Friesian calves (n = 4464) in northern Mexico (25°N).

Birth weight	Year season			
range (kg)	Spring	Summer	Autumn	Winter
26-30	6.54±0.19$^{a\ A}$	6.03±0.12$^{a\ B,C}$	6.13±0.09$^{a,b\ B}$	5.81±0.07$^{a,b\ C}$
31-35	6.32±0.08$^{a\ A}$	6.07±0.06$^{a\ B}$	6.10±0.05$^{a-c\ B}$	5.92±0.04$^{a\ C}$
36-40	6.14±0.05$^{a\ A}$	5.98±0.04$^{a\ B}$	6.08±0.04$^{a-c\ A,B}$	5.87±0.03$^{a,b\ C}$
41-45	6.14±0.06$^{a\ A}$	5.93±0.05$^{a,b\ B}$	6.14±0.05$^{a\ A}$	5.78±0.04$^{b\ C}$
46-50	5.82±0.11$^{b\ A}$	5.90±0.09$^{a,b\ A}$	5.94±0.06$^{b,c\ A}$	5.85±0.06$^{a,b\ A}$
51-55	6.02±0.13$^{a,b\ A}$	5.86±0.17$^{a,b\ A}$	6.05±0.13$^{a-c\ A}$	5.92±0.14$^{a\ A}$

$^{a-c}$Values with different superscript lowercase letters in the same column represent differences (P≤0.05) between birth weight ranges for the same year´s season (P value = 0.000). $^{A-C}$ Values with different superscript capital letters in the same row represent statistical differences (P≤0.05) between year´s season for the same birth weight range (P value = 0.000).

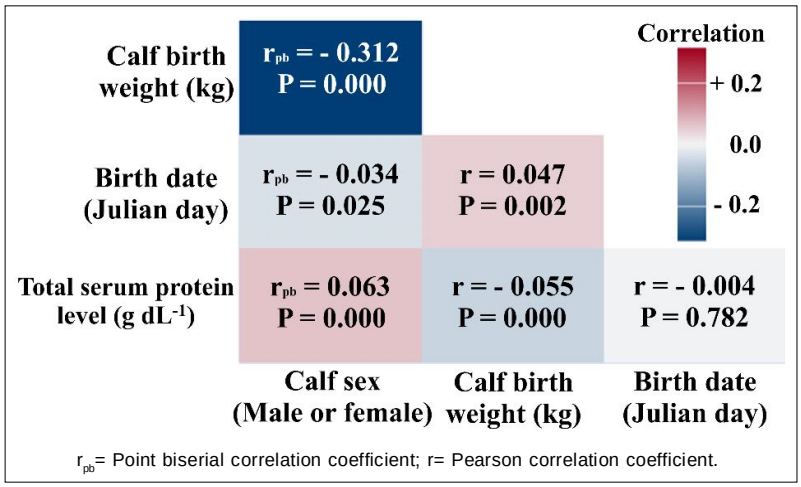


Fig 1: Correlation matrix between the variables considered in this study in Holstein-Friesian calves (n = 4464) in northern Mexico (25°N).

Table 4: Mean±mean's standard error of the sex x birth weight range in total serum protein levels (g dL^{-1}) in holstein-friesian calves (n = 4464) in northern Mexico (25°N).

Birth weight range (kg)	Sex	
	Female	Male
26-30	6.07±0.06 $^{a\ A}$	5.93±0.11 $^{a,b\ A}$
31-35	6.04±0.03 $^{a\ A}$	6.12±0.06 $^{a\ A}$
36-40	6.04±0.02 $^{a\ A}$	5.92±0.03 $^{b\ B}$
41-45	6.04±0.03 $^{a\ A}$	5.92±0.03 $^{b\ B}$
46-50	5.92±0.07 $^{a\ A}$	5.87±0.05 $^{b\ A}$
51-55	5.93±0.15 $^{a\ A}$	5.98±0.08 $^{a,b\ A}$

$^{a-c}$Values with different superscript lowercase letters in the same column represent differences (P≤0.05) between birth weight ranges for the same sex (P value = 0.028). ^{A,B} Values with different superscript capital letters in the same row represent statistical differences (P≤0.05) between sex for the same birth weight range (P value = 0.017).

(Turini *et al*., 2020). In this same sense, Aghakhani and others (2023), indicated that variables related to PIT are determined by multiple mechanisms, while BW is mainly influenced by prenatal factors.

According to Peña-Revuelta *et al*., (2024), successful PIT acquisition could be considered when TSPL are higher than a 5.5 g dL^{-1}. In addition, McGuirk and Collins (2004) proposed that the main objective of a BDU must be that at least 80% of the calves reach or exceed the TSPL aforementioned reference value (5.5 g dL^{-1}). Therefore, based on the information previously stated, the results obtained in this study can be considered successful, as 79% of the newborns reached the aforementioned reference value. In addition, other studies have reported TSPL levels of 90.2% (Peña-Revuelta *et al*., 2024) and 85.0% (Abdullahoğlu *et al*., 2019) in Holstein-Friesian calves that have reached or exceeded values of 5.5 g dL^{-1}. Considering the results obtained, the initial hypothesis is not rejected, as SB, sex and BW influence the TIP, as determined by TSPL, in Holstein-Friesian calves in the CLM.

CONCLUSION

Taken into account the geographical and environmental conditions under which the study was conducted, the research has proven that the efficiency of PIT-determined by TSPL in Holstein-Friesian calves-was influenced by BS, sex and BW, as well as by the interaction between these variables. Regarding the highest calculated TSPL, the interaction between sex and BS showed the highest values in females born in spring. Regarding the interaction between BS and BWR, the highest TSPL were recorded by calves born in spring, with a 21-25 kg BW. Regarding the interaction between sex and birth weight range, the highest levels were found in females born with a 16-20 kg BW. These findings suggest improved reproductive and management programs can be designed for this type of production unit, with the aim of enhancing calf vitality and, consequently, calf health.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

Informed consent was obtained from the owner of the animals involved in this study. All experimental procedures

and animal management used in this study were performed in accordance with international and national standards of ethics, care and animal welfare for research.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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