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Field evaluation of passive transfer in Holstein calves: Serum brix refractometry versus radial Immunodiffusion and associations with early body weight change

Evaluación de campo de la transferencia pasiva en terneros Holstein: refractometría de Brix en suero frente a inmunodifusión radial y asociaciones con cambios tempranos en el peso corporal.

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ABSTRACT

This study compared the performance of the Brix refractometer and radial immunodiffusion for assessing passive transfer immunity in Holstein calves and examined the relationship between passive transfer immunity and early body weight gain during the first 72 hours of life. A total of 136 Holstein calves that received ≥ 3 L of high-quality colostrum (Brix ≥ 22 %) within 30 minutes after birth were included. Blood samples for immunoglobulin G and Brix readings were collected 36–48 hours post-birth, and body weight was recorded at 0, 24, 48, and 72 hours. Two immunoglobulin G thresholds were applied: 24 g/L ('excellent' passive transfer immunity; Group 24) and 18 g/L ('adequate' passive transfer immunity; Group 18). Correlation analyses were performed between radial immunodiffusion and Brix values, as well as between immunoglobulin G and body weight gain indices across intervals (0–24, 0–48, 0–72, 24–48, 24–72, 48–72 hours). Significant correlations were found between Brix and radial immunodiffusion ($r = 0.804$; $P < 0.001$), confirming the reliability of the refractometer for field use. Mean immunoglobulin G concentrations differed significantly between low and high groups for both thresholds ($P < 0.001$). No significant immunoglobulin G differences were observed by gender (29.54 ± 1.26 vs. 28.04 ± 1.17 g/L; $P = 0.432$) or birth weight (≤ 38.7 vs. > 38.7 kg; $P = 0.466$). Correlations between immunoglobulin G and body weight gain were weak and nonsignificant ($P > 0.05$). body weight gain did not differ by passive transfer immunity category, although males showed slightly greater weight loss at 24–48 hours ($P = 0.026$) and higher gain at 48–72 hours ($P = 0.048$). Overall, the Brix refractometer strongly correlated with radial immunodiffusion, validating its practical use, but early body weight gain was not a reliable indicator of passive transfer immunity status.

Key words: Passive transfer immunity, immunoglobulin G, brix refractometer, radial immunodiffusion, early body weight gain.

RESUMEN

Este estudio comparó el rendimiento del refractómetro Brix y la inmunodifusión radial para evaluar la inmunidad de transferencia pasiva en terneros Holstein y examinó la relación entre la inmunidad de transferencia pasiva y el aumento de peso corporal temprano durante las primeras 72 horas de vida. Se incluyeron un total de 136 terneros Holstein que recibieron ≥ 3 L de calostro de alta calidad (Brix ≥ 22 %) en los 30 minutos posteriores al nacimiento. Se tomaron muestras de sangre para determinar los niveles de Inmunoglobulina G y Brix entre 36 y 48 horas después del nacimiento, y se registró el peso corporal a las 0, 24, 48 y 72 horas. Se aplicaron dos umbrales de Inmunoglobulina G: 24 g/L (inmunidad de transferencia pasiva «excelente»; grupo 24) y 18 g/L (inmunidad de transferencia pasiva «adecuado»; grupo 18). Se realizaron análisis de correlación entre los valores de inmunodifusión radial y Brix, así como entre los índices de Inmunoglobulina G y peso corporal temprano en los distintos intervalos (0-24, 0-48, 0-72, 24-48, 24-72 y 48-72 horas). Se encontraron correlaciones significativas entre Brix y inmunodifusión radial ($r = 0,804$; $P < 0,001$), lo que confirma la fiabilidad del refractómetro para su uso en el campo. Las concentraciones medias de Inmunoglobulina G difirieron significativamente entre los grupos bajo y alto para ambos umbrales ($P < 0,001$). No se observaron diferencias significativas en Inmunoglobulina G por sexo (29,54 ± 1,26 frente a 28,04 ± 1,17 g/L; $P = 0,432$) o peso al nacer (≤ 38,7 frente a > 38,7 kg; $P = 0,466$). Las correlaciones entre IgG y peso corporal temprano fueron débiles y no significativas ($P > 0,05$). El peso corporal temprano no difirió según la categoría de inmunidad de transferencia pasiva, aunque los machos mostraron una pérdida de peso ligeramente mayor a las 24-48 horas ($P = 0,026$) y una ganancia mayor a las 48-72 horas ($P = 0,048$). En general, el refractómetro Brix se correlacionó fuertemente con el inmunodifusión radial, lo que valida su uso práctico, pero el peso corporal temprano no fue un indicador fiable del estado de inmunidad de transferencia pasiva.

Palabras clave: Inmunidad por transferencia pasiva, inmunoglobulina G, refractómetro Brix, inmunodifusión radial, ganancia de peso corporal temprana.

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INTRODUCTION

Newborn calves are born agammaglobulinaemic because the placenta of ruminant animals prevents the passage of immunoglobulins (Ig) through the placenta [1]. Within the first hours (h) after birth, high-quality colostrum must be administered in sufficient quantities and at the right time, followed by its absorption in the intestines, to ensure passive immune protection. The immunity thus provided is called passive transfer immunity (PTI) [2].

The failure of this process (failure of passive transfer, FPT) continues to be alarmingly prevalent worldwide. Calves experiencing FPT are more likely to develop diarrhoea and pneumonia before weaning, have a higher risk of death, and exhibit slower average body weight gain (BWG), all of which result in measurable significant economic losses for producers [3].

The reference method used to determine PTI is the measurement of serum immunoglobulin G (IgG) levels by radial immunodiffusion (RID). However, RID analyses are expensive and require 24-h incubation of samples. Therefore, portable digital or optical Brix refractometers that determine serum total protein (STP) are preferred on farms. STP measured in the first days of life shows a strong correlation with serum IgG ($r \approx 0.80$), but blood collection, equipment costs, and variable cut-off values still limit its widespread use [4 , 5].

Early BWG changes may provide a non-invasive, labour-efficient alternative screening tool. Experimental studies have shown that milk intake and physical activity during the first five days of life predict calves' subsequent growth and health performance, suggesting that very early weight change may reflect both nutritional adequacy and overall vitality. However, there appears to be insufficient research in this area [6].

This study aims to investigate the potential use of early-stage body weight (BW) change as a non-invasive and labour-efficient alternative screening method for assessing passive transfer success in Holstein calves. The current literature indicates that milk intake and physical activity levels during the first five days of life play a decisive role in calves' subsequent growth performance and health status [6].

These findings support the idea that body weight change during the first week of life can be used as both an indirect indicator of colostrum intake and nutritional adequacy and an early marker of overall vitality and health. However, there are insufficient studies validating early weight change as a screening tool in relation to passive immunity status in calves [6]. This research aims to fill this gap in literature.

MATERIALS AND METHODS

Ethical approval

The study protocol was conducted with the approval of the Ankara University Local Ethics Committee for Animal Experiments, dated 07.08.2024 and numbered 2024-12-95.

Study design and animal material

This study was conducted on healthy Holstein calves born between January and July 2025 at the same dairy farm. A total of 136 calves were included in the study, whose umbilical cords were disinfected with povidone-iodine (7 %) within ≤ 2 h of birth and who were fed ≥ 3 L of high-quality colostrum (Brix ≥ 22 %) within the first 30 minutes (min).

Calves that were stillborn, aborted, died within the first 72 h, had diarrhoea or pneumonia, were weak and unable to stand on their own, had no suckling reflex, or were born by caesarean section or difficult delivery were excluded from the study.

Group formation

The calves, whose passive transfer immunity levels were determined by serum Brix refractometer (Milwaukee, MA871, USA), were divided into two groups: low and high. The BW changes at 24, 48, and 72 h, the effects of gender, and the serum IgG levels of the groups determined by the Brix refractometer were compared.

To assess the passive immunity status of calves, blood was collected via a single jugular vein puncture at 36-48 h of age. At the same time, BW was measured at birth (0 h) and at 24, 48, and 72 h using an electronic scale with ± 0.1 kg accuracy (Kontartı, 75-125cm/ 600 kg, Türkiye).

When forming the groups, a preliminary assessment using a Brix refractometer determined that there were too many animals above the passive immunity transfer cut-off value due to environmental and herd management success at the farm where the samples were collected. In order to group the samples, calf sera with high and low Brix values were subjected to RID analysis using the Brix value as a reference, and groups were formed based on the IgG levels obtained from the RID results. Two different groupings were made using threshold values of 18 g/L and 24 g/L, with values below the threshold forming the low group and values above the threshold forming the high group.

Laboratory analyses

Fresh serum samples were analysed using a portable digital refractometer (Milwaukee MA871, USA; measurement range 0–30 % Brix). Passive transfer success was defined as a cut-off value ≥ Brix %7.8 [7].

The IgG level in the same samples was measured using a radial immunodiffusion kit (JJJ Diagnostic, Kent Laboratories Inc., USA) [8]. An IgG value < 10 g/L was used as the threshold value for passive transfer insufficiency (PTI), an IgG level ≥ 18 g/L was used as a reference for adequate PTI, and a level ≥ 24 g/L was used as a reference for excellent PTI [9].

Statistical analysis

In the study, the Shapiro–Wilk normality test was first applied to evaluate the distribution characteristics of the data for statistical evaluation. For intergroup comparisons, the independent samples t-test was used for parameters showing a normal distribution, and the Mann–Whitney U test was used for data not showing a normal distribution. The interaction between the BW group and sex on the % Brix value, IgG levels,

and weight variables was examined using two-way analysis of variance (ANOVA).

In correlation analyses, the relationships between BW changes, % Brix value, and IgG levels were calculated using Pearson or Spearman correlation coefficients. All analyses were performed using IBM SPSS Statistics version 23.0, with an alpha level set at 0.05.

Given the limitations of the study, the research was conducted on a single farm, with a limited sample size and a short follow-up period. This limits the external validity and the capacity to evaluate long-term results.

Although measurements were taken using electronic scales, variables such as weighing time, digestive tract content, and hydration status may affect the accuracy of weight data. Future studies should deepen the subject with a multi-farm design, weekly growth monitoring, and a holistic approach that includes indicators of immunity, metabolism, and gut health.

RESULTS AND DISCUSSION

In this study, two main findings regarding PTI measurement in Holstein calves within the first 72 h after birth stand out. The correlation between RID-IgG measurement using a Brix refractometer in field conditions is strong, and although the thresholds reported as 18 g/L for 'adequate' and 24 g/L for 'excellent' IgG clearly distinguish the groups, they do not show a significant correlation with early body weight changes. These results do not allow the use of early-stage body weight changes to monitor passive transfer success.

A high level of statistically significant correlation was found between serum refractometer measurement and IgG level (r = 0.804; P < 0.001, TABLE I).

TABLE I
Correlation between refractometer and IgG

	Refractometer	IgG
Refractometer	1	0.804***
IgG		1
***: P<0,001		

The Pearson correlation coefficient between Brix refractometer (%) measurements and serum immunoglobulin G (IgG; g/L) concentrations determined by the radial immunodiffusion (RID) method. IgG: Immunoglobulin G.

When examining refractometer–IgG agreement and clinical relevance, the high correlation identified between the refractometer and IgG (r = 0.804; P < 0.001) is consistent with reports in the literature. Deelen *et al.* [10] reported a correlation of r ≈ 0.93 between % Brix and RID-IgG in 397 calf sera and shared that the sensitivity-specificity balance was best at an 8.4 % threshold for % Brix. Similarly, systematic reviews emphasise that refractometer-based tests demonstrate satisfactory accuracy in detecting PTI for < 10 g/L IgG and are advantageous for herd PTI monitoring due to their operational applicability.

Findings of this study support that while IgG remains the gold standard, refractometers can also be used as a valid criterion in the field [9 , 10 , 11 , 12]. Despite a strong correlation being established between IgG and Brix refractometer, the failure of preliminary assessments using the Brix refractometer to successfully predict the 10 g/L threshold indicates that the prediction success rate for IgG thresholds below 10 and 18 g/L using the refractometer is moderate, while the prediction success rate for IgG thresholds above 25 g/L is high Akköse *et al.* [13] report that the refractometer's prediction success for IgG thresholds below 10 and 18 g/L was moderate, while prediction success for IgG thresholds above 25 g/L was quite good.

Immunoglobulin G means were expectedly high for both threshold values, and a significant difference was observed between the high and low groups (TABLES II). In the IgG measurement, the mean IgG measurement results in the low category at the ≥ 24 g/L threshold value were significantly lower than in the high category (20.70 ± 0.46 vs 35.98 ± 0.91 g/L; P < 0.001, TABLE II). A similar differentiation was observed at the ≥ 18 g/L threshold (16.00 ± 0.50 vs 31.09 ± 0.83 g/L; P < 0.001, TABLE II).

TABLE II
Serum immunoglobulin G concentrations in calves classified according to 24 g/L and 18 g/L thresholds

Parameter	Group (24)		P	Group (18)		P
	Low	High		Low	High	
IgG (g/L)	20.70 ± 0.46	35.98 ± 0.91	< 0.001	16.00 ± 0.50	31.09 ± 0.83	< 0.001

Data are presented as mean ± standard error. Serum immunoglobulin G (IgG) concentrations were measured by the radial immunodiffusion (RID) method. Calves were classified into Low (< cut-off) and High (≥ cut-off) groups based on 24 g/L and 18 g/L thresholds. Comparisons between groups were analysed using the independent samples t-test.

In this study, the low and high groups were statistically significantly differentiated for both thresholds of 18 and 24 g/L, which were determined as IgG cut-off values. This is consistent with the multi-level PTI classification proposed by Lombard *et al.* [9] (excellent ≥ 24, good 18–24.9, moderate 10–17.9, poor < 10 g/L) [9 , 14].

Correlations between serum IgG levels determined by RID and BW changes during the first 72 h were not statistically significant (all P > 0.05; TABLE III). There was a positive correlation between IgG levels and body weight levels, but this was not statistically significant.

TABLE III
Correlations between immunoglobulin G and body weight indices

		IgG	BW 0-24	BW 0-48	BW 0-72	BW 24-48	BW 24-72	BW 48-72
IgG	r	1	0.056	0.062	0.090	0.021	0.073	0.051
	P		0.522	0.479	0.300	0.807	0.401	0.558

Pearson correlation coefficients examining the relationships between serum immunoglobulin G (IgG; g/L) concentrations and body weight (BW) gain indices across different time intervals.

Although BWG were higher in the group with blood IgG levels below 18 g/L in calves during the first 72 h, the difference was not statistically significant (TABLE IV). Although BWG were higher in the first 72 h in the group of calves with blood IgG levels below 24 g/L, the difference was not statistically significant (TABLE IV).

TABLE IV
Body weight indices (kg) in calves classified according to 18 g/L and 24 g/L immunoglobulin G thresholds

Parameter	Threshold (18 g/L)		P	Threshold (24 g/L)		P
	Low	High		Low	High	
BW24-0	2.07 ± 0.74	1.11 ± 0.21	0.101	1.44 ± 0.33	1.10 ± 0.26	0.410
BW48-0	1.75 ± 0.77	0.91 ± 0.22	0.183	1.26 ± 0.34	0.84 ± 0.30	0.359
BW72-0	2.79 ± 0.71	1.82 ± 0.21	0.099	2.17 ± 0.32	1.78 ± 0.27	0.354
BW48-24	-0.32 ± 0.23	-0.20 ± 0.11	0.674	-0.18 ± 0.12	-0.25 ± 0.16	0.742
BW72-24	0.72 ± 0.22	0.71 ± 0.11	0.982	0.73 ± 0.16	0.69 ± 0.13	0.823
BW72-48	1.04 ± 0.16	0.91 ± 0.11	0.645	0.92 ± 0.12	0.94 ± 0.15	0.905

Data are presented as mean ± standard error. Body weight (BW) gain indices were calculated for calves categorized according to 18 g/L and 24 g/L serum immunoglobulin G (IgG) thresholds. IgG concentrations were measured by the radial immunodiffusion (RID) method. Groups (Low vs. High) were compared using the independent samples t-test

When the relationship between early-stage BW changes and passive immunity was observed, no significant correlation was found between IgG measurement results and 0–72-h BW gain indices. Mila *et al.* [15] reported in a study conducted on dogs that they found a significant result affecting passive transfer success between daily BW change on the second day after birth and serum IgG level [15]. Furthermore, it was considered that weighing timing, hydration status, and the amount of content in the digestive tract may have contributed to the lack of statistically significant differences in BWG between groups in the 18 and 24 g/L classifications.

Therefore, the weight change dynamics in the short term also have limited ability to reflect the biological effect of PTI. The literature indicates that the effect of PTI on clinical outcomes is more evident in the medium to long term, such as reduced morbidity and mortality, daily BWG, and advanced performance. In this context, comprehensive reviews and meta-analyses demonstrating the protective effect of strong PTI on neonatal health report a significant increase in the risk of death and disease in PTI. The finding that the study results indicated a significant weight difference between groups in the short-term follow-up is consistent with existing evidence demonstrating that the benefits of PTI become more apparent in the long term [14 , 16].

In the evaluation, two groups with birth weights below and above 38.7 kg were compared, and no statistically significant difference was observed between the groups (TABLE V). The serum IgG level of female calves aged 36-48 h was measured as 29.54 ± 1.26 g/L, while that of male calves was measured as 28.04 ± 1.17 g/L. No statistically significant difference was observed between the serum IgG levels of female and male calves (TABLE V).

TABLE V
Values immunoglobulin G g/L according to Birth Weight and Sex to calves

Parameter	Birth Weight (kg)		P	Sex		P
	≤ 38.7	> 38.7		Female	Male	
IgG (g/L)	29.46 ± 1.04	28.19 ± 1.44	0.466	29.54 ± 1.26	28.04 ± 1.17	0.432

Data are presented as mean ± standard error. Serum immunoglobulin G (IgG) concentrations were measured by the radial immunodiffusion (RID) method. The effects of birth weight (classified by 38.7 kg threshold) and sex on IgG levels were analysed using the independent samples t-test.

The findings, indicating that serum IgG levels did not differ significantly according to sex and birth weight, suggest that under optimal conditions where management procedures are standardized, colostrum management, genetics, and sex—as inherent factors influencing passive transfer adequacy are not primary determinants. Rather, the provision of appropriate colostrum remains of critical importance. Indeed, Godden et al. [14] and Weaver et al. [17] also emphasize that providing an adequate quantity of high-quality colostrum as soon as possible after birth is the most fundamental determinant of passive transfer success.

Body weight indices were similar between the two time periods in terms of sex, although a significant difference was observed between the two time periods (TABLE VI). BW showed a more pronounced short-term weight loss in males compared to females in the 24-48 range (−0.57 ± 0.22 vs −0.06 ± 0.12 kg; P = 0.026), while a higher BW increase was observed in males in the subsequent BWG 48-72 range (1.23 ± 0.18 vs 0.78 ± 0.13 kg; P = 0.048). No statistically significant gender-related differences were observed in the other indices.

TABLE VI
Body weight indices (kg) according to the sex

Parameter	Group (IgG 24)		P
	Female	Male	
BW24-0	1.20 ± 0.24	1.31 ± 0.48	0.820
BW48-0	1.14 ± 0.23	0.74 ± 0.54	0.496
BW72-0	1.92 ± 0.23	1.96 ± 0.49	0.934
BW48-24	-0.06 ± 0.12	-0.57 ± 0.22	0.026
BW72-24	0.72 ± 0.12	0.65 ± 0.19	0.754
BW72-48	0.78 ± 0.13	1.23 ± 0.18	0.048

Body weight (BW) gain indices (kg) in calves classified according to female and male calves, compared using the independent samples t-test to evaluate the effect of sex across different postnatal time intervals.

While studies in the literature report higher IgG concentrations in female calves compared to male calves [18], larger sample studies have reported no significant difference

between the sexes [19]. In the present study, the body weight difference observed in the early period (decrease between 24-48 h, recovery at 72 h), which was statistically significant, may be due to temporary variation, considering the relatively short follow-up period, limited sample size, possibility of measurement errors, and physiological fluid balance changes specific to the early postnatal period in calves. It is also thought that weight change in the first 72 h may be strongly influenced by factors that fluctuate rapidly, such as placental fluid loss at birth, thermoregulation-related efficiency loss, and individual differences in colostrum intake rate [20 , 21].

The use of IgG as a reference method and the collection of blood samples at 36-48 h is consistent with the current literature in the assessment of PTI [22 , 23]. At the same time, data obtained from a single farm reduced potential variations due to homogeneous management but limited the diversity of management procedures. The short follow-up period of 72 h may be insufficient to understand the cumulative effects of PTI or PTI on growth and health [9 , 12].

IgG and refractometer measurements can be used complementarily in the field, with IgG-based thresholds of 18 and 24 g/L reliably distinguishing calves, but PTI does not show a linear and immediate relationship with early (0–72 h) weight changes. The findings support that the diagnostic focus in PTI assessment should be on serological measurements and colostrum management processes rather than short-term body weight change dynamics [9 , 10].

CONCLUSION

As a result of this study, it is recommended that refractometer-based Brix measurements continue to be used in PTI screenings. Importantly, adequate colostrum consumption remains the cornerstone of successful passive transfer; therefore, ensuring timely intake of a sufficient volume of high-quality colostrum and maintaining proper colostrum management practices should be emphasized as a primary preventive strategy against PTI failure under field conditions. However, body weight monitoring should be considered as a complementary metric for long-term growth performance and health monitoring rather than as a short-term PTI indicator. It is thought that future studies should continue to investigate fast, low-cost, and non-invasive strategies for PTI screening under field conditions.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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