

# Evaluation of colostrum quality and passive transfer immunity in terms of heat stress and disease incidence in Holstein cattle in Central Anatolia

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## ABSTRACT

The effects of heat stress on colostrum quality, passive transfer failure, and disease incidence were investigated in a large population in order to prevent calf mortality and yield losses in Holstein cows and calves. There was a statistically significant correlation between colostrum quality and the daily temperature humidity index, 7-day average temperature stress, and average temperature humidity indexes experienced by the cows during the dry period ( $P < 0.001$ ). It was observed that passive transfer failure occurred in 21% of the calves. There was a significant positive correlation ( $P < 0.05$ ) between the relative humidity and the temperature and humidity index values of the day of birth and the calf serum brix value. A negative and significant correlation was observed between Temperature-Humidity Index (THI) and serum brix value ( $r = -10, p < 0.01$ ). It was observed that the passive transfer success and diarrhea and pneumonia that were overcome in the first 365-day period showed a negative correlation ( $P < 0.01$ ). As a result, it has been observed that the heat stress experienced by cows and calves affects colostrum quality and passive transfer success, which also affects development and protection from diseases.

## Introduction

Due to the special placental structures of cows, newborns are born with agammaglobulinemia (25). Calves need maternal Ig transferred with colostrum for the natural formation of the immune system in the neonatal period (22). In calves, macromolecules such as Ig taken with colostrum after birth are absorbed directly by intestinal epithelial cells without any change and go into circulation (10). Ideal colostrum feeding should be done by giving colostrum in sufficient quantity and quality as soon as possible from the moment the calf is born (3).

The most critical period is the neonatal period, as it is the period with the highest incidence of diseases and deaths for dairy cattle enterprises (20). Successful passive transfer immunity is required for economically sustainable herd management (1). For a successful passive immune

transfer, the calf's serum IgG concentration is expected to rise above 10 g/L between 32 and 48 hours (8). Failure of passive transfer is not a disease, but it increases the likelihood of disease and adversely affects the development of the calf (25).

It is thought that heat stress may have potential effects on colostrum quality and passive transfer success (PTS) (12, 26). Heat stress occurs when body temperature exceeds the thermoneutral range due to inadequate temperature regulation. It is evaluated using this method, as it also evaluates the effect of increasing humidity on reducing heat loss (7). An alternative methodology for evaluating cooling requirements in cattle involves the utilization of the Temperature Humidity Index (THI). This composite metric, incorporating both ambient temperature and relative humidity, has demonstrated superior efficacy

compared to the sole consideration of temperature in gauging the environmental influences on lactating cattle (5).

The aim of this study is to investigate the effects of heat stress, which may affect maternal productivity and cause calf deaths, on colostrum quality, passive transfer success, and disease incidence in calves.

## Materials and Methods

**Animals:** The animal material for this study was obtained from a professional dairy farm with a 2000 dairy cows' capacity in the Bala district of Ankara, located between 39° north latitude and 33° east latitude. The data we used in our study belongs to 1043 Holstein calves and their mothers born between June 2020 and July 2021. Stillbirth, abortion, animals that died in the first 48 hours, and animals whose animal health cards could not be accessed were excluded from the study. The cows were housed in a semi-open free-stall dairy barn, and the calves were housed in calf huts. Cows were fed a total mixed ration (soybean meal, canola meal, silage, straw, vitamin-minerals, molasses, and water). Feeding and vaccination programs during the dry period and postpartum are standardized. On the farm, newborn calves are fed the colostrum that the calf accepts to drink every 2 hours, starting within the first hour after birth. Calves that drink less than 2 liters of milk in the first feeding are fed with an oesophageal probe, and the minimum colostrum volume in the first feeding is completed to 2 liters.

**Data Collection:** The data included in the study were obtained from the witness samples recorded and stored under the newborn protocols of the farm. Date of birth, time of birth, colostrum quality, diarrhea, and cases of pneumonia in the first 365 days were recorded.

Colostrum quality was measured by trained delivery room personnel with an optic brix refractometer (ATC LYK SUR-1 Clinical Refractometer, China) with a range of 0 to 32% brix. When evaluating the results, the brix 22% value was accepted as equivalent to 50 g/L Ig density, which is considered the limit of good-quality colostrum, and below this value was classified as poor-quality colostrum (3). The blood serum taken from the calf between 32 and 48 hours after birth was removed and frozen at -20 °C. Cryo-serum samples were thawed in the laboratory and re-evaluated by a researcher using an optic brix refractometer (Index Instruments, Cambridge, UK) with a range of 0 to 32% Brix and recorded as "serum brix value." When evaluating the results, brix 8.4% was accepted as the cut-off value for PTS, and below this value was considered passive transfer failure (serum IgG < 10 g/L) (2). The birth score was evaluated according to the degree of intervention at birth. The unassisted birth of the

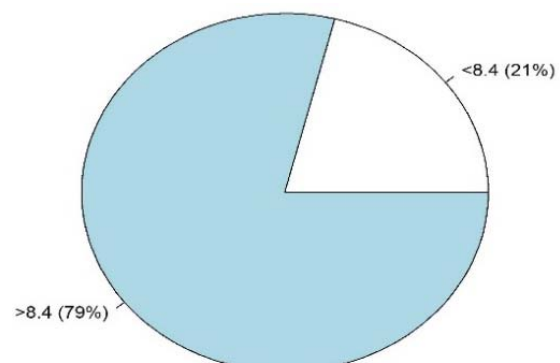
cow was numbered with 1 point. Operation caesarean section was evaluated at 5 points (16).

The temperature-humidity index (THI) was calculated by taking the lowest and highest temperature and relative humidity data from the general directorate of meteorology. It was calculated using the formula  $THI [(Temperature, Humidity) = (9/5 \times temperature + 32) - (11/20 - 11/20 \times humidity) \times (temperature - 26)]$  (19). The daily THI was calculated using the meteorological data on the day the cows gave birth. By using the calculated daily temperature and humidity indices, the average of the stress experienced by the cows during the dry period (2 months), the stress experienced during the 7 days before birth, and the 2-day stress level of the calf were calculated. The cut-off value was not used during statistical analysis to prevent data loss. However, THI was evaluated as 68–71 normal stress, 72–79 average stress, 80–89 severe stress, and 90–98 very severe stress (5).

**Statistical Analysis:** The data were analyzed with RStudio with the R 4.3 version and are presented as mean  $\pm$  SD. Because the samples did not follow a normal distribution according to the Shapiro-Wilk test ( $P < 0.05$ ), non-parametric tests were applied for statistical analysis. We used Spearman correlation to investigate the relationship between variables. A two-sample Wilcoxon test (Mann-Whitney equivalent) was used for the comparison of groups.

## Results

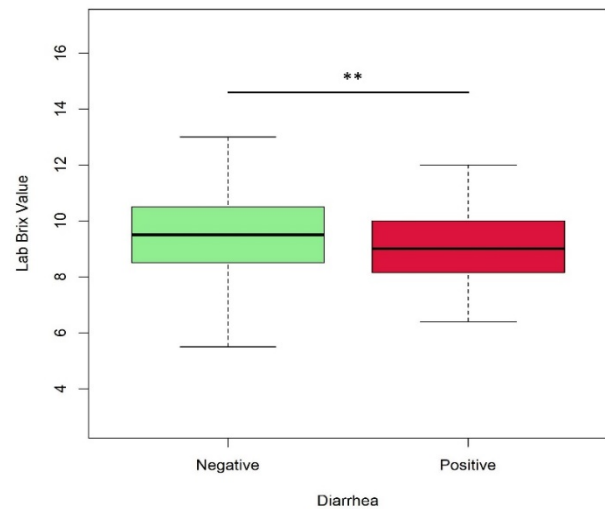
The average quality of the evaluated colostrum was calculated as brix 28.75%. 13% of the cows produced poor-quality colostrum (brix < 22%). In 21% of the calves, serum brix values were below 8.4%, which is considered the cut-off value for failure of passive transfer (FPT) (Figure 1). The factors affecting colostrum quality and passive transfer success are reported in Table 1. In the first 365 days, diarrhea was observed in 13.71% and pneumonia in 24.35% of all calves. In calves with passive transfer failure, 16.81% diarrhea and 30.97% pneumonia were observed in the first 365 days.



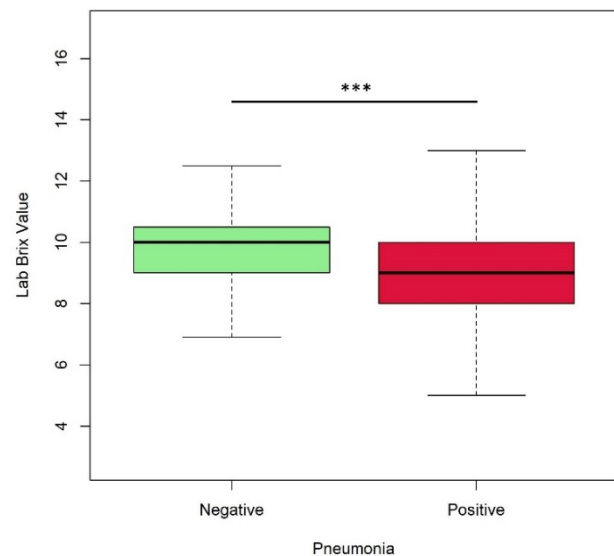
**Figure 1.** Serum Brix Value in Calves.

The minimum temperature during the study period was  $-5.4\text{ }^{\circ}\text{C}$ , and the maximum was  $36.1\text{ }^{\circ}\text{C}$ . Humidity varied between 10.5% and 95.8%. Heat stress was determined by using the temperature-humidity index formula according to the daily temperature and humidity findings to which the animals included in the study were exposed. THI ranged from 24.7 to 92.43 over the study period. According to the THI scale, 68.39% of the 1043 animals were at a normal stress level or below, 21.37% at a moderate stress level, 9.77% at a severe stress level, and 0.47% at a severe stress level. When the average THI was calculated during the dry period, 86.83% of the cows were under normal stress, 13.08% were under average stress, and 0.09% were under severe stress. There was a positive and statistically significant correlation between the temperature stress experienced by the cows during the dry period ( $P<0.001$ ), last week ( $P<0.001$ ), calving day ( $P<0.001$ ), and colostrum quality. Relative humidity had no significant effect on colostrum quality ( $P > 0.05$ ) (Table 1).

The effects of factors such as diarrhea (Table 2, Fig. 2) and pneumonia (Table 3, Fig. 3) on colostrum quality and passive transfer success in pairwise comparison have been reported.



**Figure 2.** Relationship between diarrhea and PTS. (\*  $P<0.05$ , \*\*  $P<0.01$ , \*\*\*  $P<0.001$ , ns: not significant).



**Figure 3.** The relationship between pneumonia and PTS. (\*  $P<0.05$ , \*\*  $P<0.01$ , \*\*\*  $P<0.001$ , ns: not significant).

**Table 1.** Relationships between colostrum quality, PTS, and other factors. (n: sample size, Mean: Arithmetic mean, St. Dev: Standard deviation, Q1: first quartile, Q3: third quartile, r: correlation coefficient).

	n	Mean	St. Dev.	Q1	Median	Q3	Corr. with Serum Brix value		Corr. with Colostrum Quality	
							r	P value	r	P value
Serum Brix Value	878	9.47	1.49	8.50	9.50	10.50	-	-	-0.02	0.54
Colostrum Qual.	789	28.75	3.34	27.00	30.00	31.00	-0.02	0.54	-	-
Daily THI	878	62.65	14.61	52.18	64.31	74.21	-0.10	<0.01	0.25	<0.001
Pre-natal THI (2-month)	878	57.38	10.64	48.43	53.43	68.72	-0.14	<0.001	0.36	<0.001
Pre-natal THI (7-day)	878	62.29	13.56	50.62	64.77	74.05	-0.15	<0.001	0.27	<0.001
Post-natal THI (2-day)	878	62.80	14.58	51.20	64.71	74.93	-0.10	<0.01	0.27	<0.001
Humidity	878	59.60	17.83	47.50	61.60	73.05	0.09	<0.01	-0.03	0.49

**Table 2.** Relationship between diarrhea and serum Brix value.

	Min	Q1	Median	Mean	Q3	Max.	n	St. Dev.
<b>Diarrhea</b>	5.100	8.150	9.000	9.226	10.000	16.000	127	1.524
<b>Healty</b>	2.800	8.500	9.500	9.515	10.500	17.000	751	1.482

min: Minimum, Q1: First quartile, Q3: Third quartile, Max: Maximum, n: sample size, St. Dev.: Standard deviation.

**Table 3.** Relationship between pneumonia and serum Brix value.

	Min	1st Qu.	Median	Mean	3rd Qu.	Max.	n	sd
<b>Pneumonia</b>	3.200	8.000	9.000	9.094	10.000	16.000	232	1.615
<b>Healty</b>	2.800	9.000	10.000	9.609	10.500	17.000	646	1.420

min: Minimum, Q1: First quartile, Q3: Third quartile, Max: Maximum, n: sample size, St. Dev.: Standard deviation.

## Discussion and Conclusion

In our study, a significant, positive correlation was detected between the heat stress exposed to cows and colostrum quality (Table 1). There is no significant effect of relative humidity on colostrum quality ( $P > 0.05$ ). There are doubts about the effect of heat stress on colostrum quality. Zentrich et al. (28) reported a negative correlation between colostrum quality and heat stress in a study of 2500 Holstein Friesian cows. Nardone et al. (18) reported in a study that they investigated the effect of temperature on colostrum quality and found that cows that are exposed to heat stress have lower colostrum quality. Consistent with our study, Gulliksen et al. (11) reported that colostrum increased during the period when seasonal temperatures increased. Additionally, Nardone et al. (18) reported that the amount of colostrum decreased in cows exposed to high temperatures. It is thought that the effect of heat stress on colostrum quality can be explained by the decrease in the amount of colostrum due to heat stress (18), the decrease in the amount of colostrum causing an increase in IgG concentration, as in low milk yielding cows (14), and as heat stress increases, the amount of IgG passing into the colostrum increases by increasing the permeability in the blood vessels due to the effect of vasodilation (21).

Heat stress, which we determined with the average THI values of the dry period, 7 days before birth and 2 days after birth, was compared with calf serum brix values, from which we obtained information about the passive transfer success of calves. A negative correlation was determined between prenatal 2-month THI ( $P < 0.001$ ), prenatal 7-day THI ( $P < 0.001$ ), postnatal 2-day THI ( $P < 0.01$ ), and calf serum brix value (Table 1). Tao et al. (23) reported a significant decrease in serum IgG ( $P = 0.03$ ), total protein ratio ( $P < 0.01$ ), and absorption efficiency ( $P < 0.01$ ) in calves born to cooled and high temperature-exposed cows (24). In a similar study by Laporta et al. (15), it was reported that the IgG ratio and

absorption efficiency measured at 24 hours were significantly ( $P < 0.05$ ) lower in calves of cows exposed to high temperatures before birth. This is thought to be caused by impairments in intestinal surface area and absorption rather than colostrum quality (6). There was a positive correlation between the heat stress to which the calf was exposed on the day of birth and the serum brix value obtained at 32–48 h, and it was statistically significant.

No statistically significant result was observed between colostrum quality and serum brix value. Colostrum quality is important for PTS, but for successful passive transfer, colostrum delivery rate and colostrum quantity are as important as quality (9). It has been reported that the second colostrum administration within the first 12 hours in newborns leads to an increase in serum Ig levels at 24–48 hours (17). Jester et al. (13) reported serum IgG levels of 38.6 mg/mL and 45.6 mg/mL, respectively, in a study in which 4 L of colostrum was administered to calves immediately after birth as a whole and divided into two applications. Similarly, in this study, calves received colostrum as much as they needed immediately after birth and were fed every 2 hours. Therefore, although colostrum quality and serum protein concentrations were not significant, this is thought to be the reason for the high PTS of the herd.

When the incidence of diarrhea and pneumonia in PTS was evaluated, it was observed that there was a positive correlation and statistically significant relationship between serum brix value and diarrhea ( $P < 0.01$ ) and pneumonia ( $P < 0.001$ ). Consistent with these results, Caffarena et al. (4) reported that serum IgG and serum protein concentrations were lower and statistically significant in groups with diarrhea. Again, in accordance with our results, Windeyer et al. (27) found passive transfer failure in 32% of 2874 calves. It was reported that the probability of pneumonia before 5 weeks was 13% in those without PTF, while this rate increased to 18% in

those with PTF. Successful passive transfer immunity was found to be effective in protecting against diarrhea and pneumonia in the early stages of life and afterward.

As a result, it was observed that heat stress determined by THI data had an effect on colostrum quality and serum brix value, which is an indicator of passive transfer success in calves. In addition, in this study, it was determined that PTS directly affected the diarrhea and pneumonia recovery rates in the first 1-year period. It has been observed that there is a positive and significant relationship between heat stress and colostrum quality, but this is actually due to the increase in concentration, as in low milk-yielding animals. It has been understood that cows should be protected against heat stress during and after the postpartum period in order to reduce the incidence of disease and increase the survival rate of newborns. Measures should be taken by farms to reduce the heat stress of cows and calves. It is thought that new studies on this subject should be supported, and breeders should be educated.

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No financial support was received.

### Conflict of Interest

The authors declared that there is no conflict of interest.

### Author Contributions

Design: HK and MG; Control/Supervision: HK, MG; Data Collection and / or Processing: HK, MG; Analysis and / or Interpretation: HK, MG; Literature Review: HK, MG Writing the Article: HK, MG; Critical Review: HK, MG.

### Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

### Ethical Statement

The present study was approved by the Animal Research Ethics Committee of the University of University of Ankara (Ethics approval number: AÜHADYEK, number 2022-21-184).

### Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

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