



## Evaluation of passive immunity absorption in Holstein Heifers

### Avaliação da absorção de imunidade passiva em Bezerras da Raça Holandesa

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#### ABSTRACT

Colostrums considered of good and very high quality are considered colostrums with a BRIX of  $\geq 25\%$ , so the first weeks of a neonate's life are considered critical, showing a greater susceptibility to diseases. In this sense, the objective was to evaluate the quality of colostrum and the transfer of passive immunity in Holstein heifers. The experiment was carried out on a milk producing property, located in the county of "Olho D'água das Flores", Alagoas, from October 2021 to April 2022, where 181 crossbred heifers were used. To measure the quality of the colostrum, an optical BRIX refractometer was used, if it showed a quality lower than 25% BRIX, this colostrum was corrected and offered to the calf. The collected blood was stored in a tube without anticoagulant and left to rest until the serum was obtained, later, a drop of this serum was applied to the refractometer prism and, similarly to colostrum, a reading was performed to determine the concentrations of total proteins. For Pearson's correlation study, correlation coefficient analysis ( $r$ ) was performed, assuming intervals of  $-1$  (negative linear association) and  $1$  (positive linear association). Serum protein showed a negative correlation with the time of colostrum supply after birth ( $r = -0.16$ ) and positive with calf weight at birth ( $r = 0.15$ ). The adequate program of colostrum and evaluation of transfer of passive immunity allied to the proper management collaborate for the success in the productive indexes of the properties.

#### RESUME

Colostros considerados de boa e altíssima qualidade são considerados colostros com um BRIX de  $\geq 25\%$ , com isso as primeiras semanas de vida de um neonato é considerada crítica, manifestando uma maior susceptibilidade às doenças. Neste sentido, objetivou-se avaliar a qualidade do colostro e a transferência de imunidade passiva em bezerras da raça holandesa. O experimento foi realizado em uma propriedade produtora de leite, localizada no município de Olho D'água das Flores, Alagoas, no período de outubro de 2021 a abril de 2022, onde foram utilizadas 181 bezerras mestiças. Para aferição da qualidade do colostro, utilizou-se o refratômetro de BRIX óptico, caso demonstrasse qualidade inferior a 25% BRIX esse colostro era corrigido e ofertado a bezerra. O sangue coletado foi armazenado em um tubo sem anticoagulante e deixado em repouso até a obtenção do soro, posteriormente, uma gota desse soro foi aplicado ao prisma do refratômetro e, de modo semelhante ao colostro, foi realizado uma leitura determinando-se as concentrações de proteínas totais. Para o estudo de correlação de Pearson foi realizado análise de coeficiente de correlação ( $r$ ), assumindo intervalos de  $-1$  (associação linear negativa) e  $1$  (associação linear positiva). A proteína sérica apresentou correlação negativa com o tempo de fornecimento do colostro após o nascimento ( $r = -0,16$ ) e positiva com o peso da bezerra ao nascer ( $r = 0,15$ ). O programa adequado de colostragem e avaliação de transferência de imunidade passiva aliado ao manejo adequado colaboram para o sucesso nos índices produtivos das propriedades.

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## Introduction

The first weeks of a newborn's life is considered critical, manifesting a greater susceptibility to diseases. According to Bittar and Paula (2014), a colostrum of good and very high quality are those that present a BRIX higher than 21% and 31% respectively, values lower than 21% indicate a lower quality of colostrum, with this it's not recommended its supply to calves. Azevedo et al. (2015), points out that most of the proteins consumed through colostrum are immunoglobulins and their quantification should take place within 48 hours after birth.

Hulbert and Moisé (2016) reported that maternal antibodies from colostrum remain in the circulatory system of calves until the first 3 weeks of life, but according to Heinrichs and Jones (2003) from the fifth day of life the passive immunity acquired from colostrum decreases, at the same time that there is still no complete maturation of the active immune system of the calf, this being the critical period for the development of diseases.

The quality of colostrum is determined by its concentration of immunoglobulins, mainly IgG. These being acquired through the contact of the mother with pathogens or via vaccination predicted in the dry period. Given this, it's essential to ensure the supply of colostrum, being possible to perform tests to verify its quality and absorption efficiency (RODRIGUES, 2012).

Allied to what was said earlier, it should be noted that there is an individual variation capable of influencing the absorptive efficiency of colostrum, but the factors related to it are not yet very clear (HALLERAN et al., 2017). Apparent Efficiency of IgG Absorption (AEA) is based on calf weight, colostrum volume offered, and IgG concentrations in colostrum and neonate serum. Ghoreishi et al. (2015) compared the effect of oral supply of substances that alter gastrointestinal motility (cisapride, betanechol and erythromycin) against the apparent absorption of Igs. In this study, they concluded that the oral supply of cisapride (a substance that accelerates motility) increased the absorption of colostrum via the gastrointestinal tract, demonstrating that the rate of abomasal emptying influences the passage of IgG to the small intestine, its absorption site.

Remembering that to be successful in raising calves and feeding management of neonates must take into account several factors such as: correct management of pregnant females, postpartum management providing a dry and clean stall, milking and supply of colostrum to the calf in the shortest possible time, because its composition changes over time and the absorption of immunoglobulins is gradually reduced after birth.

The success in transferring passive immunity to calves also depends on other factors such as: volume ingested, interval between birth and ingestion, sanitary quality of colostrum and absorption capacity, according to Silper et al. (2012). A quick and inexpensive method to evaluate the quality of colostrum is the quantification of soluble solids through the BRIX grade refractometer, which can be found in optical and digital versions.

For the use of this instrument it's necessary to calibrate it with distilled water, followed by the application of a drop of colostrum in the prism of the refractometer, performing the reading that infers the concentrations of immunoglobulins G (IgG), through the estimation of the percentage of total solids present in the content (BITTAR and PAULA, 2015).

Finally, the quality of colostrum is directly related to the concentration of Igs (in particular, IgG) and absence of bacteria, and may vary depending on factors such as the number of lactations, breed, dry period of the cow, which can influence both the volume and concentration of IgG in colostrum (LORENZ et al., 2011). A quality colostrum is defined by having IgG concentrations above 50 g/L (JEZEK et al., 2012; MCGUIRK; COLLINS, 2004).

The mean IgG concentration in the colostrum of Holstein cows in their first lactation is around  $42.3 \pm 11$  g/L (JEZEK et al., 2012), and Franklin et al. (1998) found an average of 77.6 g/L and Bertier et al. (2015) found an average of 65.1 g/L. Study conducted by Chigerwe et al. (2008) evaluated colostrum from 160 Dutch cows and observed that about 32% had production of a colostrum of poor quality and ( $< 50$  g/L), values close to the 29.1% found by Bertier et al. (2015).

The objective of this study was to evaluate the quality of colostrum and the transfer of passive immunity in Holstein calves by measuring serum protein.

## Materials e Methods

The work was carried out at the YBY Poranga Farm, located in the county of "Olho d'água das Flores", Alagoas, Brazil, precisely in the western region (Latitude:  $9^{\circ} 32' 12''$  South, Longitude:  $37^{\circ} 17' 39''$  West). This region is considered as a medium backwoods, due to the long periods of rainfall and its dry and hot climate.

The material collected for the analysis were: the colostrum of the cows and the blood of the female calves all of the Dutch breed. Blood was collected 24 hours after colostration totaling 181 females in all, from October 2021 to April 2022. The collected blood was stored in a tube without anticoagulant and left to rest until the serum is obtained, later, a drop of this serum is applied to the prism of the refractometer and, similarly to colostrum, the reading is made by determining the concentrations of total proteins.

For the execution of the study was used distinct models of optical refractometers, one capable of observing the contents of total soluble solids through the BRIX grade refractometer, thus evaluating the quality of the colostrum, and can also evaluate the Serum Protein through the use of the protein refractometer.

Parturitions ranged from one to five per day, the matrices were dried sixty days before parturition and taken to the pre-partum paddock thirty days before parturition (Figure 1), remaining in the environment until parturition, with no separation between heifers and cows. From the moment they are removed for the pre-partum period, a specific diet, vaccinations

against verminosis, neonatal diarrhea and pneumonia are started, providing newborns with antibodies from ingested colostrum.

Table 1 - Composition of colostrum, transitional milk and whole milk.							
		Number of milkings (cows milked twice a day).					
		1	2	3	4	5	11
Component	Unit	Colostrum	Transitional Milk				Whole Milk
Total Solids	%	23.9	17.9	14.1	13.9	13.6	12.9
Proteins	%	14	14	8.4	5.1	4.2	4
Casein	%	4.8	4.3	3.8	3.2	2.9	2.5
Antibodies	%	6	4.2	2.4	0.2	0.1	0.09
Fat	%	6.7	5.4	3.9	4.4	4.3	4
Lactose	%	2,7	3.9	4.4	4.6	4.7	4.9
Minerals	%	1.11	0.95	0.87	0.82	0.81	0.74
Vitamin A	UI/dl	295	113			74	34
Minnesota University.				Author: Cintia Almeida.			
Source: Nutival Technical Department.				Published in the year of 2011.			

The difference between colostrum and transitional milk is in accordance with their composition between secretions. As can be seen in table 1. It's worth mentioning that, it's only considered colostrum the first milking of the matrix, the next milkings is considered transitional milk.

After delivery the matrices were directed to milking for the removal of colostrum and then directed to the lots of newly calved, they remain in the period of one month until the change of lot, in which it's directed to a specific lot according to its production.

**Figure 1.**

*Cows in the precalving phase.*



Source: SILVA, J. R. (2021).

The calves were separated from their mothers soon after birth, thus performing the healing of the navel, later they were taken to the calf and weighed. The ingestion of colostrum was done artificially only once, through a bottle or nasogastric tube (Figure 2), the amount provided was standardized regardless of the weight of the calf.

**Figure 2.**

*Colostration via nasogastric tube.*



*Source: SILVA, J. R. (2021).*

The matrices were milked soon after parturition, in order to collect colostrum. Then, the total soluble solids content was evaluated using a BRIX grade refractometer, in order to correct it if it presented a content below 25%. For plasma collection, blood samples were collected after 24 hours of colostration of the neonate, by puncturing the jugular vein with vacuum collection tubes, using the same for the clot accelerator blasted on the wall, for the coagulation process. Then the samples were kept at rest to obtain the serum.

To determine the analyses by means of refractometry, the BRIX and Serum Protein refractometer was used, in which it was calibrated according to its recommendations, that is, with the aid of distilled water using one to two drops to adjust the zero scale in the refractive prism. A drop of the sample was applied for each reading with the aid of a 3 ml syringe (Figure 3), the drop was placed under the surface of the prism, then the lid was closed for observation against the light, which then the value deposited on the meniscus between the blue and white shades were recorded.

**Figure 3.**

*Measurement of total protein contents with refractometer.*



*Source: SILVA, J. R. (2022).*

For the determination of immunoglobulins G, it was observed in the colostrum samples, using the refractometer with BRIX scale (Figure 4), which determined the percentage of total solids, where the values above 25% of BRIX degree indicated good quality colostrum, and values lower than 25% were considered of inferior quality being corrected with colostrum powder.

**Figure 4.**

*Internal BRIX scale properly calibrated with zero refraction.*



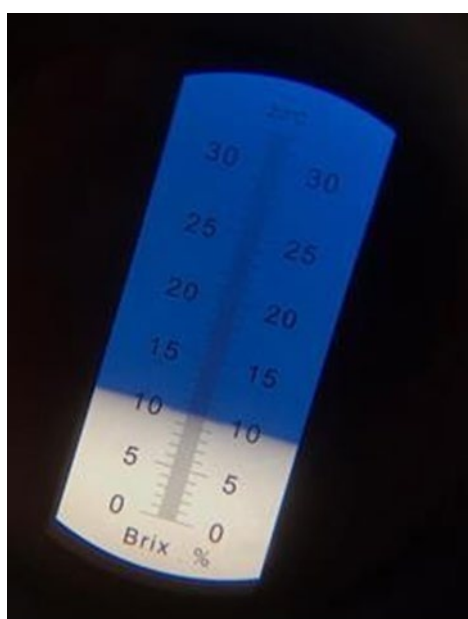
*Source: SILVA, J. R. (2022).*

The determination of serum protein contents was performed after resting the blood samples until the serum was obtained, for 60 to 120 minutes. Next, a drop was added to the prism of the serum protein refractometer for reading. These values were used to estimate passive immunity transfer, so that plasma samples that presented values greater than 5.5 g dl<sup>-1</sup> (Figure 5) indicate success in passive immunity transfer, in which success in colostrum can be observed.

Regarding values between 5.0 and 5.4 g dl<sup>-1</sup>, moderate passive immunity transfer; and values lower than 5.0 g dl<sup>-1</sup> demonstrate failure in the transfer of passive immunity (BITTAR and PAULA, 2014).

**Figure 5.**

*Concentration of total proteins presents in plasma.*



*Source: SILVA, J. R. (2022).*

Data were analyzed using Student's t test and Pearson's correlation analysis, at a minimum significance level of 0.05, using the Rcommander package of the statistical program R.

## **Results e Discussions**

Table 2 shows that the concentration of total proteins, present in the blood plasma of the calves evaluated, had a positive result in relation to the quality of colostrum (BRIX 25%) and the quantity offered to the calves, thus obtaining a high absorption of IgG. Overall, the data showed low standard deviations and coefficients of variation (CV% < 20%), indicating good uniformity of the data. All plasma samples for analysis indicated their success, presenting in all samples total protein concentrations greater than 5.5g dl<sup>-1</sup>.

**Table 2.**

*Mean, standard deviation, coefficient of variation (CV) and number of observations (n) of the variables serum protein, amount of colostrum offered, BRIX degree (%) and calf weight at birth.*

<b>Variable</b>	<b>Mean ± Standard deviation</b>	<b>CV (%)</b>	<b>N</b>
Serum protein (%)	9.93 ± 0.93	9.40	181
Quantity (litros)	4.0 ± 0.04	1	181
BRIX Grade (%)	25.28 ± 0.85	3.4	181
Calf weight (Kg)	35.08 ± 3.61	10.3	181

Coefficient of variation (CV); Number of samples (N).

*Source: The author (2022).*

According to Soberon et al. (2012) the calves that received four liters of colostrum had higher feed efficiency than the other treatments that did not receive enough colostrum. Calves with failure in passive immunity transfer (FTIP), had reduced milk and fat production in the first lactation and delay in age at first delivery (Godden et al. (2009). For each unit of IgG greater than 12 mg/ml there is a 9 kg increase in the metabolizable energy of milk (Osaka et al., 2014).

According to Jones et al. (2004) demonstrated that calves fed maternal colostrum had higher feed efficiency, greater weight gain and lower age at puberty than those treated with colostrum replacement of serum derivatives. Reber et al. (2008) found that the use of acellular colostrum characteristic of frozen colostrum, can compromise the development of the immune system of calves compared to the use of fresh colostrum.

Serum protein showed a negative correlation with colostrum delivery time after birth ( $r = -0.16$ ) and positive correlation with calf weight at birth ( $r = 0.15$ ). For Pearson's correlation study, correlation coefficient ( $r$ ) analysis was performed, assuming intervals of -1 (negative linear association) and 1 (positive linear association). Data were analyzed using the PROC GLM and PROC CORR of the Software Statistical Analysis System University (SAS 2015) at a level of 5% probability by the "t" test. Freezing is a conservation method that prevents significant bacterial growth, which allows for greater conservation of the product. There is no effect of freezing on Ig concentrations, but it has an effect on cellular immunity, since the ice crystals formed are responsible for breaking the membrane of the cells present in the colostrum (Kryzer et al., 2015).

According to Raboisson et al. (2016) failure to transfer passive immunity (FTIP) is the main contributing factor to calf mortality, being associated with 39 - 50% of Dutch calf mortality (Bartier et al., 2015). Table 3 shows that there was no significant difference in relation to fresh or frozen colostrum, maintaining its properties without alteration.



**Table 3.***Serum protein according to milk status (fresh/frozen) and animal category (cow/heifer).*

<b>Serum protein</b>	<b>Mean ± Standard deviation</b>	<b>CV (%)</b>	<b>N</b>
<b><u>Milk</u></b>			
Fresh	9.93 ± 0.91 <sup>a</sup>	9.13	147
Frozen	9.91 ± 1.05 <sup>a</sup>	10.63	34
<b><u>Animal category</u></b>			
Heifer	10.16 ± 0.91 <sup>a</sup>	8.95	59
Cow	9.81 ± 0.93 <sup>b</sup>	9.45	122

Coefficient of variation (CV); Number of samples (N).

*Source: The author (2022).*

FTIP is associated with high risks of mortality, decreased health and longevity of calves, which directly impacts costs during the breeding phase of these animals. The correct realization of colostrum (quality, quantity and optimal delivery time) is responsible for increasing the size, width and number of intestinal villi, the depth of the crypts and thickness of the mucosa, the synthesis of enzymes from the brush edge, glucose uptake, intestinal DNA synthesis, antioxidant activities against oxidative stress and growth factors present in serum. According to Yang et al. (2015) state that the factors that assist after birth in the establishment of the immune defense mechanism and the antioxidant system, reducing morbidity and mortality.

The epithelial cells of the gut form a potent physicochemical barrier that limits microbial growth and access to the surface of the intestine. They may also recruit leukocytes to complement barrier function or to participate in immune responses. The development of gut-associated lymphoid tissues (GALTs) is started before birth.

However, the maturation of this system and the recruitment of IgA cells and activated T cells occurs after birth and is dependent on signals derived from the microbiota; these signals influence intestinal epithelial cells and dendritic cells, modulating the nature and intensity of T cells. Most of this cell population is made up of macrophages, neutrophils, T and B lymphocytes, with immunoreactive and immunomodulatory capacity, which contribute to rotavirus protection.

Animals receiving colostrum containing maternal leukocytes develop more rapidly antigen-presenting cells, which are essential for the development of the acquired immune response to pathogens and vaccines. Leukocytes increase lymphocyte response to non-specific mitogenic substances by increasing phagocytosis and the ability to kill bacteria, and increase Ig formation in calves (Bensussan and Routhiau 2010; Langel et al., 2015).

## Final considerations

In view of the results obtained, we can consider that, for animals in a state of experiment, there was variation in the amount of immunoglobulins absorbed by the calves, showing a superior result for the supply of colostrum (BRIX 25%) in greater quantity and better quality.

Further studies can be considered to define exactly which ideal parameters of colostrum, in quality, quantity and supply period and its arrangement, to achieve peaks of absorption of immunoglobulins (IgG) by calves and their profitability for execution in the various models of production and rearing of calves.

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