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Short communication

Use of digital Brix refractometer to estimate total protein levels in Santa Inês ewes' colostrum and lambs' blood serum

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ABSTRACT

The current study aimed to estimate the total protein levels in Santa Inês ewes' colostrum and in lambs' blood serum by digital refractometry to determine the transfer of passive immunity to lambs under farm conditions. Nineteen ewes and 29 lambs were used. Ewes' colostrum and lamb blood serum samples were collected at 6, 12, 24 and 48 h postpartum to measure the total serum protein by digital Brix refractometer. The confirmation of lambs immunological status was performed by the zinc sulphate turbidity (ZST) test on blood samples collected from lambs 12 and 24 h postpartum. Total protein concentration in colostrum significantly decreased between 6 and 12 h postpartum ($p < 0.05$). This reduction was accompanied by a significant increase in lambs' total serum protein concentration ($p < 0.05$), indicating the occurrence of passive immunity transfer in the first 12 h of life of these newborns. These findings were confirmed by the ZST test, which showed a positive reaction in 82.75 % and 75.86 % of the samples after 12 and 24 h, respectively. In conclusion, digital Brix refractometer proved to be a valuable tool for estimating total protein concentration in Santa Inês ewes' colostrum and in lamb blood serum under farm conditions.

1. Introduction

The syndesmochorial placenta of the sheep prevents the transfer of maternal immunoglobulins to the fetus during pregnancy (Vejlsted, 2010), making the ingestion of colostrum in the first 24 h of life indispensable (Alves et al., 2015). Its protective function is ensured by the presence of a complex mixture of bioactive substances and antimicrobial proteins such as IgG, lactoferrin, lactoperoxidase, lysozyme, and proline-rich polypeptides (Séverin and Wenshui, 2005). When immunity transfer failure occurs, it is relatively common for neonatal septicemia, pneumonia and diarrhea to appear, consequently, there is an increase in neonatal morbidity and mortality rates, leading to large economic losses (Costa et al., 2013).

According to Vatankhah (2013), the immunoglobulin levels found in lamb serum are directly associated with the immunoglobulin concentrations present in ewe's colostrum. Thus, the knowledge about the immunoglobulin content in colostrum as well as in lamb blood serum is essential to determine correct actions aimed at reducing lamb mortality. To date, only few studies have been conducted to develop strategies for optimizing passive immunity transfer in lambs, especially in the Northeast region of Brazil. Some of the factors that have contributed to this reality are the techniques commonly employed to determine passive immunity transfer. Most of the studies developed with this theme considers radial immunodiffusion and ELISA as gold standard techniques for this purpose (Gelsinger et al., 2015; Alves et al., 2015). However, both techiniques are costly and require trained laboratory technicians, making them unfeasible to perform under farm conditions (Deelen et al., 2014).

A reliable and inexpensive solution to this problem has been proposed by Quigley et al. (2013). These authors demonstrated that digital

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and optical refractometers can be successfully used to assess colostrum quality in cattle, with emphasis on Brix refractometry, which allows the evaluation of colostrum quality and the identification of adequate passive immunity transfer. Using this methodology, a correlation between low Brix values % and failure of transfer of passive immunity has been demonstrated in calves (Morrill et al., 2012; Deelen et al., 2014). In swine, Brix refractometer has been shown to be a cheap, fast and satisfactory instrument for estimating Ig concentration, allowing differentiation between good- and poor-quality colostrum (Hasan et al., 2016).

In previous study, Alves et al. (2015) evaluated the colostrum composition of Santa Inês ewes and the passive transfer of immunity to lambs using indirect ELISA test and enzymatic colorimetric kits, respectively. However, to our knowledge, studies using Brix refractometry to evaluate total protein levels in Santa Inês ewes' colostrum and in blood serum of lambs reared in a semi-extensive system in Northeastern Brazil have not yet been developed. Therefore, the objective of this study was to obtain information on the passive immunity transfer of lambs under farm conditions by estimating the total serum protein levels in Santa Inês ewes' colostrum and in the blood serum of lambs by digital Brix refractometer.

2. Material and methods

2.1. Animal experimentation ethics committee

All procedures performed in this experiment were conducted in accordance with the guidelines of the Animal Experimentation Ethics Committee of the Federal University of Piauí (protocol number 175/16). Nineteen clinically healthy adult Santa Ines ewes were estrous-synchronized and all lambs were born in the same period. Throughout the gestation and postpartum period, the ewes were managed during the day in Tanzania-grass pastures and in native pasture. At the end of the afternoon they were placed in collective pens and received soybean meal concentrate, corn, urea, salt and water ad libitum. Near delivery, all ewes were transferred to maternity paddocks and monitored during and up to 48 h after lambing. During the colostrum feeding period, lambs were kept with ewes under natural conditions of suckling conformed described by Hernández-Castellano et al. (2015).

2.2. Sample collection and processing

Ewes' colostrum samples were collected by hand milking at 6, 12, 24 and 48 h after lambing and stored in 1.5 ml polyethylene microtubes. To obtain blood serum, blood samples from 29 lambs were collected by puncturing the jugular vein 6, 12, 24 and 48 h after birth using a 25 × 8 mm needle (BD Vacutainer, BD Medical, Juiz de Fora, Brazil) and vacuum blood collection tubes (Vacutainer, Shandong Weigao Medical Group Polymer Co. Ltd., Hong Kong, China). All blood samples were centrifuged at 4200 x g for 10 min. Immediately after obtaining the blood serum, a part of it was used for Brix refractometer it and the remaining part was stored in 1.5 mL polyethylene microtubes and frozen at -20 °C for subsequent ZST test.

2.3. Brix refractometry and zinc sulphate turbidity (ZST) test

Total protein concentration in colostrum as well as in lambs' blood serum were measured at room temperature and at field using a digital Brix refractometer with a range 0–85 % Brix (Hannah HI 96801 Instruments Inc., Woonsocket, Rhode Island, USA). Refractometer calibration as well as samples reading were performed according to the manufacturer's instructions.

To perform the ZST test, 350 mg/L zinc sulfate solution ($ZnSO_4$-$7H_2O$) was used according to procedures established by Hogan et al. (2016). This test was performed at 12 and 24 h after lambing as an immunological diagnosing. The absence of turbidity or the presence of weak turbidity is indicative of failure of transfer of passive immunity (Hogan et al., 2015).

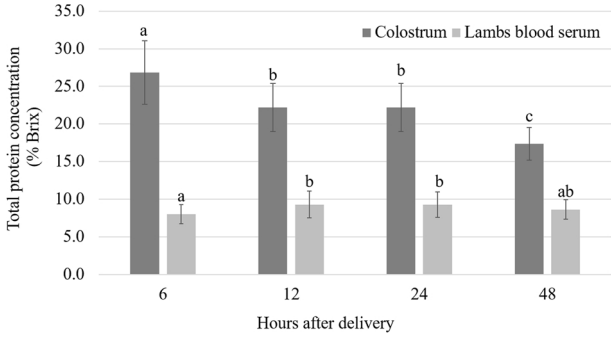

Fig. 1. Total protein concentration measured by digital Brix refractometer in Santa Inês ewes' colostrum and lambs blood serum at 6, 12, 24 and 48 h after lambing. Different letters indicate significant differences between the different evaluation moments within the same evaluated parameter ($p < 0.05$). Error bars represent standard deviation of the mean value.

2.4. Statistical analysis

Descriptive analysis of the results obtained in the ZST test was performed. The other data were submitted to normality analysis (Kolmogorov-Smirnov test) and variance homoscedasticity (Levene). Total protein concentrations found in colostrum and in lambs' blood serum at different times after lambing (6, 12, 24, 48 h) were submitted to ANOVA and means were compared by Tukey test. Data were analyzed using a statistical program (Bio Estat, version 5.0) and significance was attributed to $p < 0.05$.

3. Results

The total protein concentration in ewes' colostrum and in lamb blood serum performed by Brix refractometry are presented in Fig. 1. The results revealed that in the colostrum there was a significant reduction in the percentage of total proteins ($p < 0.05$) between 6 and 12 h after lambing, remaining it constant between 12 and 24 h, followed by a significant reduction after 48 h ($P < 0.05$).

On the other hand, a significant increase ($p < 0.05$) was observed in the percentage of total protein dosed in lambs' blood serum between 6 and 12 h after lambing, indicating the occurrence of passive immunity transfer in the first 12 h of life of these newborns. From 12 h after delivery, serum protein levels remained constant in all lambs.

To confirm the transfer of passive immunity indicated by Brix Refractometry, the ZST test was performed 12 and 24 h after lambing. This analysis revealed a positive reaction in 82.75 % of the samples after 12 h and in 75.86 % of the samples after 24 h after delivery. In addition, it was verified that the reaction of the ZST test was positive only in samples that presented values % for total serum protein higher than 100 mg/dL.

4. Discussion

In this study it was observed that Brix refractometry provided a satisfactory estimate of total protein present in Santa Inês ewes' colostrum as well as in the blood serum of lambs at different times after lambing. Similar results were described by Deelen et al. (2014) using Brix refractometer to estimate IgG concentration in calf blood serum samples. In another study, Quigley et al. (2013) estimated the colostrum IgG levels of cows in the first milking using Brix refractometer. Recently, Hasan et al. (2016) demonstrated that Brix refractometer can be used at farm level to analyze the colostrum in the first hours after delivery and to indicate IgG content classification, allowing for

improved management of lactating sows and newborn piglets.

The concentration of IgG in colostrum is crucial for the acquisition of passive immunity (Kielland et al., 2015). In the current study, we observed a gradual decrease in total protein concentration in colostrum at first 48 h after lambing. These results are in agreement with previous studies conducted by Devillers et al. (2011), who found that IgG levels decreased significantly within 10–12 hours postpartum and after 24 h postpartum IgG levels became unsatisfactory. We also showed that the blood serum of lambs had a significant increase in total protein concentration from 12 h of life. These findings provide strong evidence that Brix refractometer has successfully detected the occurrence of passive immunity transfer.

The ZST test consists of precipitation of high molecular weight proteins in solution causing turbidity of the sample (Hudgens et al., 1996). In this study, the ZST test was used to confirm the results obtained by Brix refractometry. The same samples obtained from lambs' blood serum analyzed by digital Brix refractometry at 12 and 24 h after lambing were examined using the ZST test. We observed that the increase of total protein concentration in the blood serum of lambs previously identified by Brix refractometry were confirmed by ZST test in 82.75 % and 75.86 % of the samples after 12 and 24 h, respectively. These findings confirming the occurrence of high passive immunity transfer rate in our experimental conditions. There is a consensus in the literature that immunoglobulins absorption from colostrum by the intestinal epithelium occurs in the first 24 h of life of the newborn (Alves et al., 2015).

These results demonstrate that Brix refractometer can be used as a practical and inexpensive instrument to evaluate the occurrence of passive immunity transfer of lambs under farm conditions. These results are consistent with those reported by Deelen et al. (2014). According to these authors, Brix has a high correlation with serum IgG levels in calves. Similarly, Morrill et al. (2012) and McCracken et al. (2017) demonstrated that Brix values % can be used to identify failures in passive immunity transfer in calves.

5. Conclusion

Digital Brix refractometer has been showed to be a valuable tool for estimating the total protein concentration present in Santa Inês ewes' colostrum as well as in lambs' blood serum. However, it is recommended that further studies be conducted on this subject in order to identify the exact cutoff point that can be detected by Brix Refractometry. This would minimize errors in estimating passive immunity transfer, thereby reducing mortality rates in newborns lambs.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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References

Alves, A.C., Alves, N.G., Ascari, I.J., Junqueira, F.B., Coutinho, A.S., Lima, R.R., Pérez, J.R.O., De Paula, S.O., Furusho-Garcia, I.F., Abreu, L.R., 2015. Colostrum composition of Santa Inês sheep and passive transfer of immunity to lambs. J. Dairy Sci. 98, 3706–3716. https://doi.org/10.3168/jds.2014-7992.

Costa, J.N., Silva, D.F.M., Lima, C.C.V., Souza, T.S., Araújo, A.L., Costa Neto, A.O., Almeida, M.M.O., 2013. Falha da transferência de imunidade passiva em cordeiros mestiços (Santa Inês x Dorper) e estudo do proteinograma do nascimento até o desmame. Brazilian J. Vet. Res. Anim. Sci. 50, 114–120. https://doi.org/10.11606/issn.2318-3659.v50i2p114-120.

Deelen, S.M., Ollivett, T.L., Haines, D.M., Leslie, K.E., 2014. Evaluation of a Brix refractometer to estimate serum immunoglobulin G concentration in neonatal dairy calves. J. Dairy Sci. 97, 3838–3844. https://doi.org/10.3168/jds.2014-7939.

Devillers, N., Le Dividich, J., Prunier, A., 2011. Influence of colostrum intake on piglet survival and immunity. Animal 5, 1605–1612. https://doi.org/10.1017/S175173111100067X.

Gelsinger, S.L., Smith, A.M., Jones, C.M., Heinrichs, A.J., 2015. Technical note: comparison of radial immunodiffusion and ELISA for quantification of bovine immunoglobulin G in colostrum and plasma. J. Dairy Sci. 98, 4084–4089. https://doi.org/10.3168/jds.2014-8491.

Hasan, S.M.K., Junnikkala, S., Valros, A., Peltoniemi, O., Oliviero, C., 2016. Validation of Brix refractometer to estimate colostrum immunoglobulin G content and composition in the sow. Animal 10, 1728–1733. https://doi.org/10.1017/S1751731116000896.

Hernández-Castellano, L.E., Suárez-Trujillo, A., Martell-Jaizme, D., Cugno, G., Argüello, A., Castro, N., 2015. The effect of colostrum period management on BW and immune system in lambs: from birth to weaning. Animal 9, 1672–1679. https://doi.org/10.1017/S175173111500110X.

Hogan, I., Crowe, B., Doherty, M., Fagan, J., Kennedy, E., Conneely, M., Lorenz, I., 2016. Optimisation of the zinc sulphate turbidity test for the determination of immune status. Vet. Rec. 178, 169. https://doi.org/10.1136/vr.103401.

Hogan, I., Doherty, M., Fagan, J., Kennedy, E., Conneely, M., Brady, P., Ryan, C., Lorenz, I., 2015. Comparison of rapid laboratory tests for failure of passive transfer in the bovine. Ir. Vet. J. 68, 1–10. https://doi.org/10.1186/s13620-015-0047-0.

Hudgens, K.A., Tyler, J.W., Besser, T.E., K.D, 1996. Optimizing performance of a qualitative zinc sulfate turbidity test for passive transfer of immunoglobulin G in calves. Am. J. Vet. Res. 57, 1711–1713.

Kielland, C., Rootwelt, V., Reksen, O., Framstad, T., 2015. The association between immunoglobulin G in sow colostrum and piglet plasma. J. Anim. Sci. 93, 4453–4462. https://doi.org/10.2527/jas.2014-8713.

McCracken, M.M., Morrill, K.M., Fordyce, A.L., Tyler, H.D., 2017. Technical note: evaluation of digital refractometers to estimate serum immunoglobulin G concentration and passive transfer in Jersey calves. J. Dairy Sci. 100, 8438–8442. https://doi.org/10.3168/jds.2017-12847.

Morrill, K.M., Conrad, E., Polo, J., Lago, A., Campbell, J., Quigley, J., Tyler, H., 2012. Estimate of colostral immunoglobulin G concentration using refractometry without or with caprylic acid fractionation. J. Dairy Sci. 95, 3987–3996. https://doi.org/10.3168/jds.2011-5104.

Quigley, J.D., Lago, A., Chapman, C., Erickson, P., Polo, J., 2013. Evaluation of the Brix refractometer to estimate immunoglobulin G concentration in bovine colostrum. J. Dairy Sci. 96, 1148–1155. https://doi.org/10.3168/jds.2012-5823.

Séverin, S., Wenshui, X., 2005. Milk biologically active components as nutraceuticals: review. Crit. Rev. Food Sci. Nutr. 45, 645–656. https://doi.org/10.1080/10408690490911756.

Vatankhah, M., 2013. Relationship between Immunoglobulin Concentrations in the Ewe's Serum and Colostrum, and Lamb's Serum in Lori-Bakhtiari Sheep. Iran. J. Appl. Anim. Sci. 3, 539–544.

Vejlsted, M., 2010. Comparative placentation. In: Hyttel, P., Sinowatz, F., Vejlsted, M. (Eds.), Essentials of Domestic Animal Embryology. Saunders/Elsevier, New York, pp. 104–119.