MAE Vet Fak Derg, 7 (2): 62-66, 2022
DOI: 10.24880/maeuvfd.992161

Research Article

Investigation of immunoglobulin G, lactoferrin and zinc levels in blood sera of calves fed fresh and frozen colostrum

Süleyman Şensoy[1], Şima Şahinduran[2]

[1]Vocational School of Food, Agriculture and Livestock, Burdur Mehmet Akif Ersoy University, Burdur, TURKEY
[2]Department of Internal Medicine , Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Burdur, TURKEY

Key Words:
colostrum
freezing
immunoglobulin
lactoferrin
zinc

Received : 07.09.2021
Accepted : 13.05.2022
Published Online : 31.08.2022
Article Code : 992161

Correspondence:
S ŞENSOY
(suleyman.sensoy@hotmail.com)

ORCID
S ŞENSOY : 0000-0003-2130-783X
Ş ŞAHİNDURAN : 0000-0002-7718-2020

This study was supported by Burdur Mehmet Akif Ersoy University Scientific Research Projects Commission (Project No: 0627-YL-20).

ABSTRACT

Colostrum has high nutritional values, is more easily digestible than milk, and has a comparatively higher concentration of dry matter, fat and non-fat dry matter, protein and most importantly, immunoglobulin (Ig). The most important task of immunoglobulins is to neutralize pathogens and toxins through neuttalization. Lactoferrin is a protein product of the transferrin gene family with iron binding ability. Lactoferrin serves as a major component of the secondary granules of polymorphonuclear neutrophils and is produced by epithelial cells, including those in the mammary gland. Zinc acts as a cofactor and activator of more than 300 enzymes in different metabolic pathways and is known as a biologically important trace mineral. The aim of this study is to examine whether the immunoglobulin G, lactoferrin and zinc concentrations in colostrum, which is vital for newborn calves, show a decrease tendency by freezing. Fresh colostrum was given to one group (n=12) and frozen (-20 °C) colostrum (n=12) was given to one group and blood samples were collected after 32 hours. According to the results obtained, there was no statistical difference between the groups in the initial measurements of IgG, lactoferrin and zinc values in group 1 (fresh colostrum) and group 2 (frozen colostrum). The differences between the first and second measurements among themselves in both groups were found to be statistically significant. In the second measurements between the two groups, no statistical difference was found between the values of immunoglobulin G ($p= 0.996$), lactoferrin ($p = 0.513$), zinc ($p = 0.605$).

INTRODUCTION

A large part of the dry matter in the composition of colostrum consists of immunoglobulins, and their most important task is to contain maternal antibodies that help protect the newborn against disease pathogens in the first days after birth. Colostrum helps to provide the energy required for the offspring to maintain body temperature and to expel meconium from the digestive system (Koyuncu and Karaca, 2018).

Immunoglobulin G is the main immune component in bovine colostrum and milk, but low concentrations of IgA and IgM are also present (Leyton et al., 2007). IgG is the smallest immunoglobulin group with the highest concentration (70-80 %) in the blood (Diker, 2011).

IgG protects the intestinal mucosa against pathogenic microorganisms and gives passive immunity to the newborn ruminant until his own immune system develops with colostrum. IgG antibodies express multifunctional activities, including complement activation, bacterial opsonization, agglutination, and act by binding to specific sites on the surface of most infectious agents or products by inactivating or reducing infection (Leyton et al., 2007). It is produced from B lymphocytes and plasma cells in secondary lymphoid organs. It is produced most intensively during secondary immune response (Diker, 2011).

Lactoferrin is a member of the transferrin family, including serum transferrin, melanotransferrin, and ovotransferrin. Lactoferrin is a glycoprotein capable of binding iron found in the milk of many species such as cow, human, goat, mare, and mouse. Lactoferrin plays a role in natural immunity, as well as antibacterial, antiviral, antifungal, antiprotozoal, anticarcinogenic, antioxidant, improving bone health, regulating iron absorption in the intestine, immunomodulation, anti-inflammatory, and cell growth control. In addition, it can exhibit quite a lot of biological activity, including the ability to bind and inhibit some bioactive compounds such as glycosaminoglycan and lipopolysaccharide (Yıldırım et al., 2011). Lactoferrin also plays an important role in the immune system defense mechanism. It prevents microorganisms from adhering to the host cell and prevent them from multiplying by colonization or kills them (Legrand et al., 2005).

Zinc (Zn), which has important physiological effects on plants and animals, is one of the essential trace elements (Önder and Yıldız, 2002). Zn is essential for the immune system. It acts by interacting specifically with the components of the immune system, which is a highly proliferative system

To cite this article:
Şensoy Ş, Şahinduran Ş. Investigation of immunoglobulin G, lactoferrin and zinc levels in blood sera of calves fed fresh and frozen colostrum. MAE Vet Fak Derg. 2022; 7 (2) : 62-66.

(Chasapis et al., 2012).

The aim of this study was to examine whether the immunoglobulin G, lactoferrin and Zn concentrations in colostrum, which is vital for newborn calves, show a decrease tendency by freezing.

MATERIAL and METHODS

The dams used in this study were Simmental cows in the 3rd lactation, with the same feeding and vaccination program from the same farm. The colostrum given to both groups were measured with a refractometer immediately after collection. Only colostrum with a dry matter of 24-26% were included in the study. The colostrum to be frozen were placed in storage containers and stored at -20 °C. The colostrum was thawed, and the temperature was raised to 37 °C (by dissolving in bain-marie) before feeding the calves from frozen colostrum group. It was provided to the calf within half an hour after birth. Calves in the fresh colostrum group also received colostrum within half an hour after birth. The calves in the study were divided into two groups; 1: those fed fresh colostrum (n=12), 2: those fed frozen colostrum (n=12). Blood samples were taken from all animals in both groups twice, the first before colostrum feeding immediately after birth, and the second after feeding 7.5 liters of colostrum 32 hours after birth. Blood samples from all calves were taken from *Vena jugularis externa* into vacutainers using a disposable sterile syringe. Silicone-based plastic tubes (9 ml) with clot activator were used for serum samples. The collected blood samples were first kept in a portube for 30 minutes to clot. Then, the sera were removed by centrifugation at 4000 rpm for 10 minutes. Serum samples were transferred to Eppendorf tubes (1.5 ml) with the help of automatic pipette. The tubes were stored at -20 °C until serological IgG, lactoferrin and Zn analyses were performed. IgG and lactoferrin values in blood serum were measured by Enzyme-Linked Immunosorbent Assay (ELISA) method. Bovine specific IgG and bovine specific lactoferrin ELISA kits (Biox®, Belgium) were used in the study. In biochemical analysis, Zn values were measured with Gesan Chem 200-1102422® (Italy) autoanalyzer device.

Statistical analysis: The findings obtained were evaluated using IBM SPSS 22.0 for Windows package program. Shapiro-Wilk test was used for the normal distribution of the data. Because of the normal distribution of data, in-group and between-group comparisons were made using two-way analysis of variance in repeated measurements. Multiple comparison tests with benferoni correction were used. Pearson Correlation coefficient was used to analyze the relationship between variables.

RESULTS

In the comparison of the first measurement of lactoferrin value in Group 1 (fresh colostrum) and Group 2 (frozen colostrum), there was no statistically significant difference ($p = 0.914$). In the comparison of the second measurement of lactoferrin value between the groups in Group 1 and Group 2, there was no statistical difference ($p = 0.513$). In group 1, a significant statistical ($p < 0.001$) difference was found in the comparison of the first measurement value of the lactoferrin value and the second measurement value. In Group 2, a significant statistical ($p < 0.001$) difference was found in the comparison of the first measurement value of the lactoferrin value and the second measurement value. (Table 1).

Table 1. Lactoferrin values in calves fed fresh and frozen colostrum.

	Group 1 (n=12)	**Group 2** (n=12)
	$\bar{x} \pm ss$	$\bar{x} \pm ss$
Lactoferrin 1	$328.33196.81^{Aa}$	319.93179^{Aa}
(ug/ml)	209.49 (146.81-664.90)	239.18 (168.16-754.46)
Lactoferrin 2	2375.551392^{Ab}	2525.121181^{Ab}
(ug/ml)	1719.65 (1146.30-4775.78)	1779.79 (558.87-4724.03)

There is statistical difference between columns with different lower-case superscripts ($p < 0.05$).
There is a statistical difference between rows with different upper-case letters ($p < 0.05$).

In the comparison of the first measurement of Zn value between groups, there was no statistical ($p = 0.762$) difference between group 1 and group 2. There was no statistically significant difference ($p = 0.605$) in the comparison of the second measurement of Zn value between groups in Group 1 and Group 2. In group 1, a significant statistical ($p < 0.001$) difference was found in the comparison of the first measurement value of the zinc value and the second measurement value. In Group 2, a significant statistical ($p < 0.001$) difference was found in the comparison of the first measurement value of the zinc value and the second measurement value (Table 2).

In the comparison of the first measurement of immunoglobulin value between the groups in group 1 and group 2, there was no statistically significant difference ($p=0,833$). In Group 1 and Group 2, there was no statistically significant difference ($p = 0.996$) in the comparison of the second measurement of immunoglobulin value between groups. In Group 1,

Table 2. Zinc values in calves fed fresh and frozen colostrum

	Group 1 (n=12)	**Group 2** (n=12)
	$\bar{x}$ ±ss	$\bar{x}$ ±ss
Zinc 1	130.4135.4^{Aa}	125.5841.5^{Aa}
(µg/dL)	138.50 (73.00-184.00)	129.50 (58.00-199.00)
Zinc 2	190.8337.34^{Ab}	182.6638.82^{Ab}
(µg/dL)	199.00 (135.00-257.00)	182.00 (121.00-235.00)

There is statistical difference between columns with different lower-case superscripts ($p < 0.05$).
There is a statistical difference between rows with different upper-case letters ($p < 0.05$).

a significant statistical ($p < 0.001$) difference was found in the comparison of the first measurement value of the Immunoglobulin value and the second measurement value. In Group 2, a significant statistical ($p < 0.001$) difference was found in the comparison of the first measurement value of the Immunoglobulin value and the second measurement value. (Table 3)

A positive correlation was found between lactoferrin and Zn (rho = 0.411; p = 0.046). No relationship was found between immunoglobulin and Zn (rho = -0.315; p =0.134). No relationship was found between lactoferrin and IgG (rho = -0.350; p = 0.094).

and Atasever, 2005). This period is limited to the first 24-48 hours after birth (Aydoğdu, 2014).

In a study conducted, serum IgG concentrations of calves receiving fresh colostrum and receiving frozen colostrum were examined and no statistical difference was found between IgG concentrations according to the results of the study (Costa et al., 2017). According to the results of this study, no statistical difference was found between IgG concentrations. In this respect, it is in parallel with our study. However, unlike our study, it has been reported that calves receiving frozen colostrum for 30 days experience severe diarrhea compared to calves receiving fresh colostrum. No diarrhea was found in the calves in both groups in our study up to one month old. In another

Table 3. Immunoglobulin values in calves fed fresh and frozen colostrum

	Group 1(n=12)	**Group 2**(n=12)
Immunoglobulin1	4.891.91^{Aa}	5.082.39 ^{Aa}
(ngr/mL)	4.30 (2.96-8.36)	4.98 (1.56-8.34)
Immunoglobulin2	64.2710.69^{Ab}	64.2415.10^{Ab}
(ngr/mL)	64.02 (44.35-82.96)	70.57 (25.03-80.21)

There is statistical difference between columns with different lower-case superscripts ($p < 0.05$).
There is a statistical difference between rows with different upper-case letters ($p < 0.05$).

DISCUSSION

A quality colostrum allows the development of the defense system against pathogens that newborn calves may encounter in the first days of life. The immune systems of the offspring of animals that do not have high quality colostrum or whose colostrum cannot be used on the grounds that they have disease cannot develop sufficiently. The resulting weakness in the immune system increases the risk of disease and death in calves (Aydoğdu, 2014). Immunoglobulins provide passive immunity until the newborn's own immune system develops and protects the intestinal mucosa against pathogens (Leyton et al., 2007). Immunoglobulins are absorbed by the process of some specialized cells in the small intestine called "pinocytosis". These cells leave their place to basal cells over time (Erdem

study, a group of calves were fed colostrum stored at +4 °C and another group was fed with colostrum frozen at -20°C (Holloway et al., 2001). In this study, no statistically significant difference was found between the IgG concentrations in the blood serum of calves fed fresh or frozen colostrum from the same dam. As a result of the study, the suggestion that frozen colostrum can be used as a source of IgG for calves was supported. The results of this study are similar to the results we obtained with IgG values.

In another study, serum IgG values were compared in calves fed fresh colostrum and a commercial colostrum supplement (Holloway et al., 2002). Significantly higher serum IgG concentrations were detected in calves fed fresh colostrum compared to calves fed colostrum supplement. This study showed

that IgG in fresh colostrum is absorbed more efficiently than IgG found in colostrum supplements.

In addition, according to the results of our study, no statistically significant difference was observed in the IgG values in the blood serum of calves fed with fresh and frozen colostrum at the 32nd hour after birth.

Lactoferrin is an iron-binding protein that forms the first defense mechanism against infections and inflammation. Lactoferrin, a multifunctional and important immunoregulatory protein, is a component of breast and lacrimal secretions, seminal and synovial fluids, and plasma and neutrophil granules. Although lactoferrin is present in plasma, its amount is significantly lower than in milk (Lönnerdal and Iyer, 1995). Tsuji et al. (1990) compared the amount of colostrum lactoferrin in cattle with different yield directions in their study. While the average amount of colostrum lactoferrin in dairy cattle was 2 mg/mL, it was found to be 0.5 mg/mL in beef cattle. While the amount of lactoferrin in colostrum is affected by the lactation number of dairy cattle, it has not been reported in beef cattle. Lakritz et al., (2000) compared the IgG and lactoferrin values in the blood serum of calves in two groups fed with pasteurized colostrum and frozen colostrum in their study. As a result of the study, it was revealed that the lactoferrin value in the blood serum of calves receiving pasteurized colostrum was lower than that of calves receiving frozen colostrum. In this study, it was determined that pasteurization method at 76 °C destroyed colostrum proteins. According to the results of our study, no statistically significant difference was observed in the lactoferrin values in the blood serum of calves fed fresh and frozen colostrum 32 hours after birth.

Zn, which is in the structure of many enzymes, is a necessary micro element for normal growth, calf development, and reproductive functions in mature animals (Elmasoğlu, 2008). Any microelement deficiency is seen during the pregnancy of cattle negatively affects the development of the fetus and calf health. It has been proven that microelements cross the placenta and breast barrier. Adequate micro element saturation in pregnant animals has been found to be important for the needs of the young during intrauterine and postnatal development. In addition, microelements also affect the milk and colostrum quality. The concentration of zinc in the blood of newborn calves is significantly higher than that of their dams, which means that the calf organism can accumulate Zn during intrauterine development (Pavlata et al., 2004). Arcagök et al., (2013) investigated the relationship between iron deficiency and blood Zn level in childhood. A statistically significant positive correlation was found between Zn levels and iron levels. This study explains the positive relationship between Zn, which has a high iron affinity, and lactoferrin in our study. It was determined that serum Cu and Zn concentrations were significantly lower in calves with diarrhea compared to healthy calves (Elmasoğlu, 2008).

CONCLUSION

In this study, blood IgG, lactoferrin, and Zn levels in calves fed fresh and frozen colostrum were compared. In the light of the results obtained, the IgG concentration (ngr / ml) in fresh colostrum (64.27 ± 10.69), frozen colostrum (64.24 ± 15.10) (p = 0.996), lactoferrin concentration (ug / ml) in fresh colostrum (2375.55 ± 1392.81), frozen colostrum (2525.12 ± 1181.34) (p = 0.513), Zn concentrations (µg / dL) in fresh colostrum (190.83 ± 37.34), frozen colostrum (182.66 ± 38.82) (p = 0.605) were observed. When the three parameters between the two groups were compared separately, the differences were not found to be statistically significant in any of them. This study demonstrated the potential applicability of colostrum taken from dams who have high quality colostrum and who do not have any health problems in calves of cows that do not have high quality colostrum, or calves that cannot receive their dam's colostrum due to health problems in their mothers, and stored by freezing.

DECLARATIONS

Ethics Approval

This research was carried out on the basis of the permission of Mehmet Akif Ersoy University Local Animal Ethics Committee dated 16.10.2019 and numbered 571.

Conflict of Interest

The authors declare that there have no conflict of interests.

Consent for Publication

Does not need a publication consent.

Author Contributions

In all sections of the final article, each author contributed equally.

REFERENCES

1. Arcagök B, Özdemir N, Yıldız İ, Celkan T. Çocukluk çağında demir eksikliğinin kan çinko düzeyi ile ilişkisi. Türkiye Çocuk Hast Derg. 2013: 56, 63-70.

2. Aydoğdu U. Sütçü İneklerde Kolostrum Kompozisyonu ve Kalitesinin Buzağı Pasif İmmunite Etkileri. Doktora Tezi Selçuk Üniversitesi Sağlık Bilimleri Enstitüsü. Konya. 2014

3. Chasapis CT, Loutsidou AC, Spiliopoulou CA, Stefanidou ME. Zinc and human health an update. Arch. Toxicol. 2012; 86, 521–534

4. Costa JFDR, Novo SMF, Baccili CC, Sobreira NM , Hurley DJ , Gomes V. Innate immune response in neonate Holstein heifer calves fed fresh or frozen colostrum. J. Res. Vet. Med., 2017; 115, 54-60.

5. Diker KS. Bağışıklığın Yapısal Unsurları Carlı KT (ed). Temel Veteriner Mikrobiyoloji ve İmmunoloji. Anadolu Üniversitesi WebOfset Tesisleri, Eskişehir. 2011. p. 121-137

6. Elmasoğlu İO. Akut ishalli buzağılarda serum demir, bakır ve çinko konsantrasyonlarının değerlendirilmesi. Yüksek Lisans Tezi T.C. Adnan Menderes Üniversitesi Sağlık Bilimleri Enstitüsü, Aydın. 2008

7. Erdem H, Atasever S. Yeni Doğan Buzağılarda Kolostrumun Önemi. OMÜ Zir. Fak. Der., 2005: 20, 79-84.

8. Holloway NM, Tyler JW, Lakritz J, Carlson SL, Holle J Serum immunoglobulin G concentrations in calves fed fresh and frozen colostrum. J. Am. Vet. Med., 2001: 219, 357-359.

9. Holloway NM, Tyler JW, Lakritz J, Carlson, SL, Tessman RK, Holle J. Serum Immunoglobulin G Concentrations in Calves Fed Fresh Colostrum or a Colostrum Supplement. J. Vet Intern Med., 2002: 16, 187–191.

10. Koyuncu M, Karaca M. Buzağılarda Yaşam Güçünün Anahtarı 'Kolostrum'. J. Anim. Prod., 2018: 59, 67-78.

11. Lakritz J, Tyler JW, Hostetler DE, Bataklık AE , DM Dokumacı DM , JM Holle et al. Effects of pasteurization of colostrum on subsequent serum lactoferrin concentration and neutrophil superoxide production in calves. Am. J. Vet. Res., 2000: 61, 1021-1025

12. Legrand D, Elass E, Marangoz M, Mazurier J. Lactoferrin: a modulator of immune and inflammatory responses. Cellular and Molecular Life Sciences 2005: 62, 2549–2559

13. Leyton WG, David EJC, Don EO, Indyk HE. Analysis of bovine immunoglobulin G in milk, colostrum and dietary supplements: a review. Anal. Bioanal. Chem., 2007: 389, 93–109.

14. Lönnerdal B, Iyer S. Lactoferrin: molecular structure and biological function. Ann. Rev. Nutr., 1995; 15, 93-110.

15. Önder F, Yıldız S. Çinko ve bakır yetersizliğinin bağışıklık sistemine etkileri. Kafkas Üniv. Vet. Med. J., 2002: 8, 183-187.

16. Pavlata L, Pechova A, Dvorak R. Microelements in Colostrum and Blood of Cows and their Calves during Colostral Nutr. Acta Vet. Brno, 2004: 73, 421-429.

17. Tsuji S, Hirata Y, Mukai F Ohtagaki S. Comparison of lactoferrin content in colostrum between different cattle breeds. J. Dairy. Sci., 1990: 73, 125-128.

18. Yıldırım Z, Tokatlı M, Öncül N, Yıldırım M. Laktoferrinin Biyolojik Aktivitesi. Akademik Gıda, 2011: 9, 52-63.