

IMMUNOGLOBULIN CONCENTRATION CHANGES IN DAIRY COWS COLOSTRUM DURING STORAGE OF FROZEN

Eihvalde Indra, Kairiša Daina, Zagorska Jeļena, Grāmatiņa Ilze

Latvia University of Agriculture, Lielā iela 2, Jelgava, LV – 3001, Latvia

Abstract

Colostrum is the first natural food for the newborn calf, it provides passive immunity to the calf. The aim of research was to estimate immunoglobulin concentration changes in colostrum during storage of frozen (6 months). The research was carried out at Latvia University of Agriculture, in dairy farm of Research and Study Farm, from December 2010 to March 2011. Colostrum samples (50 mL⁻¹) from 23 dairy cows were collected during one hour after calving. They were frozen minus 18 °C and stored for 1 week and 6 months. Immunoglobulin A, G and M concentration was determined in colostrum by Colostrometer and Turbidimetric method, but protein content by Kjeldahl method. Average protein content was 15.2%, but total immunoglobulin concentration in colostrum after 1 week was 17.9 ± 1.17 mg mL⁻¹ and after 6 months was significantly lower 10.2 ± 0.68 mg mL⁻¹ ($p < 0.05$), it significantly decreased by 7.7 mg mL⁻¹ or 1.7 times. Immunoglobulin M after six months decreased by 90%, immunoglobulin A by 40% and immunoglobulin G by 32%.

Key words: immunoglobulin, protein, frozen colostrum

INTRODUCTION

One of the most significant tasks to get a high – quality herd is healthy rearing of calf. Colostrum is essential for calves because they are born without any appreciable immunity. Colostrum is the first and irreplaceable food for calf, it consists of biologically active components which provide health and growth of new – born calf. Colostrum contains antiviruss, antibacterial and antiinflammation influence and provides passive immunity. (Quigley, 2001). One of the most important antibacterial components are immunoglobulins, they are specific proteins of immune system, which are transferred from blood serum into colostrum produced by cow before calving. Immunoglobulins fulfill protective functions in the body, they fight with disease-causing microorganisms, neutralize toxins and viruses (Korhonen et al., 2000; Georgiev, 2008). Immunoglobulins A, M and G are found in cow's blood serum and colostrum (Barrington et al., 2000). The dominant immunoglobulin class is immunoglobulin G, (Quigley, 2001), its content in colostrum in average is 49.6 – 89.9 mg mL⁻¹ or 75 - 80% of the total Ig concentration. Immunoglobulin G protects an organism against viruses, bacterias, fungi and toxic substances (Korhonen et al., 2000; Georgiev, 2008). Immunoglobulin A protects an organism against microorganisms in the body enters through the body surface covered with mucous membranes, such as through the nose, eyes, lungs and digestive tract. Immunoglobulin A content is 3.2 – 6.2 mg mL⁻¹ or 13 – 15% of the total amount of immunoglobulins. (Korhonen, et al., 2000). Immunoglobulin M fights for pathogen-organisms got in blood serum or other body fluids. Immunoglobulin M content is 3.7 – 6.1 mg mL⁻¹ or 6 - 10% of the total immunoglobulin concentration in colostrum (Korhonen et al., 2000). Bovine colostrum contents of five main components which is water, proteins, fats, lactose, and mineral salts. Content of colostrum oscillates from 8 – 21%, moreover 80% it is whey protein and 20% casein. Total casein consists of three fractions: α - casein and κ - casein, immunoglobulins are the main components of whey protein, which forms 90%, other components β - lactoglobulin, α - lactoglobulin and lactoferrin (Quigley, 2001). Casein content in colostrum is less than milk but amino acids - lysine,

methionine, valine, threonine, and tryptophan are more in colostrum. After 24 hours in mammary gland, most of the proteins are casein and albumin (Ontsouka, Bruckmaier, 2003).

An important factor that influences colostrum quality (immunoglobulin concentration) is the age of the cow. Heifers have poor quality colostrum while older cows have the best quality colostrum. Another very important factor is milking stage. In general, colostrum immunoglobulin content is halved with each successive milking, therefore the first milking colostrum has twice the immunoglobulin content of the second milking colostrum (Quigley, 2001). High quality colostrum should be collected and frozen from three and older lactation cows, which have antibacterial components formed during cows life. Using frozen colostrum from older cows, it is recommended to feed to calves in the first 24 hours, but primiparous colostrum – in the next days. Colostrum with immunoglobulin concentration higher 50 mg mL⁻¹ is recommended to freeze and feed in case of need, when cows colostrum is with blood presence, high somatic cell count or immunoglobulin concentration lower than 30 mg mL⁻¹ (Barrington et al., 2000). Fresh colostrum can be stored till 5 days 4 °C (Giles et al., 2002). Frozen colostrum is recommended to store no more than one year (Merrick's, 2005), after thawing colostrum is recommended to feed during one hour, because bacterial pollution gets greater (Quigley, 2001). To collect high quality colostrum, cows teats have to be cleaned before milking, otherwise bacteria which get into colostrum increase fast and create high bacterial pollution. Colostrum should be milked from the cow into a clean pail. The colostrum should then be allowed to cool to room temperature and tested with a colostrometer as described above. Milked colostrum has to be filled in 1 – 2 litres sterile plastic bottles and frozen till minus 18 °C (Elizondo – Salazar et al., 2010). There are disadvantages in freezing colostrum because it destroys white blood cells. These cells, leukocytes, present in colostrum at the level of 1,000,000 per milliliter. These cells directly defend the calf against infections, and they stimulate her immune cells to be more effective antigen killers and to reproduce more rapidly (Barrington, Parish, 2001). To keep biologically active component concentration colostrum has to be thawed in 38 – 40 °C water bath, fast thawing in high temperature can denature proteins. It is recommended to choose a lower power microwave oven to thaw colostrum, to keep higher immunoglobulin concentration in colostrum (Merrick's, 2005). By using different thawing methods, immunoglobulin concentration can be changed in colostrum (Jones et al., 1987).

MATERIALS AND METHODS

The research was carried out at the Latvia University of Agriculture (LUA), in dairy farm of the Research and Study Farm 'Vecauce', in a loose housing system with 530 cows, of which 430 were milking cows. The cows during the dry period were kept tethered in the farm. The research is carried out from December 2010 to March 2011. Twenty three Latvian brown breed dairy cows which are calved from December to February were used in this research.

The research has included cows that colostrum immunoglobulin concentration was > 50 mg mL⁻¹. Different lactation cows were used in this research, 2 of which were first - lactation cows, 7 - second, 8 - third, 3 - fourth-, 1 – fifth and 2 - sixth lactation cows. Two samples of colostrum were obtained from each cows, it was collected by manual milking, setting immunoglobulin concentration and protein content. Colostrum samples (50 mL⁻¹) were collected during one hour after calving. Quality of colostrum was estimated using a Colostrometer by company DeLaval (Biogenics – Colostrometer, 1980). Before evaluation, colostrum was heated till 22 °C. The Colostrometer evaluates colostrum's quality (or immunoglobulin concentration) as superior – 50 to 140 mg mL⁻¹, moderate – 30 to 50 mg mL⁻¹, or inferior < 30 mg mL⁻¹. These quality levels are also coded by colour on the Colostrometer as green, yellow, and red (Fleenor, Stott, 1980).

All 46 samples were frozen in minus 18 °C and 23 samples were stored for 1 week and others 23 samples were stored for 6 months. Colostrum samples were analysed for immunoglobulins A, M, and G concentration and protein content. According to total immunoglobulin concentration (determined by Colostrometer) in colostrum cows were classified into three groups: 1st group – 50 till 80 mg mL⁻¹, 2nd group – 81 till 100 mg mL⁻¹ and 3rd – 101 till 123 mg mL⁻¹. Obtained results about immunoglobulins G, A and M concentration and protein content in colostrum groups are classified considering cows age: cows from the first and second lactation and third and older lactation.

Immunoglobulins concentration in colostrum was determined by Turbidimetric method, but protein content was determined by Kjeldahl method (LVS EN ISO 8968-1:2002).

Statistical analysis of the data was performed by using Microsoft Excel. Descriptive statistics is performed to analyse results: arithmetical mean and Standard Error of arithmetical mean ($\bar{x} \pm s_{\bar{x}}$). The data were subjected to one – way analysis of variance (ANOVA) to determine the significance of differences of means among immunoglobulin concentration. Linear correlation coefficient is used to determine results connection. Statistical analysis of results was performed by using a T- test. Statistical significance ($p < 0.05$) was indicated with different alphabet letters in superscript.

RESULTS AND DISCUSSION

Immunoglobulin concentration determined by colostrometer for research cows was in amplitude from 50 – 125 mg mL⁻¹, in average 88.2 ± 3.67 mg mL⁻¹, which comply with high quality colostrum. The average use of research cows was 3 lactations. Scientists have confirmed that immunoglobulin concentration in older cow's colostrum is higher than in younger cow's colostrum (Liu et al. 2009, Kehoe et al., 2011), it is also confirmed in our research. Primiparous cows in herd are displaced shortly before calving and specific antibodies are not sufficiently formed in their blood serum against viruses, bacteria and parasites or existing microflora in herd (Quigley, 1995, Dowlen et al., 1995).

Immunoglobulin G was 67% from the common immunoglobulin content, but in other scientists researches it was 75 till 80% (Korhonen et al., 2000; Geogiev, 2008). Immunoglobulin M concentration in colostrum was 18%. It is confirmed in research that immunoglobulin M concentration is 6 – 10% (Korhonen et al., 2000). It is confirmed in researches that raised immunoglobulin M in colostrum can point to recent animals contact with illness inhibitor (Coico et al., 2003). Immunoglobulin A concentration in colostrum was 15%, which complies to results of other scientists – 13 till 15% (Korhonen et al., 2000).

In spite of the fact that immunoglobulin concentration that was determined with Turbidimetric method, was low, gained results about immunoglobulins proportion comply with results published in other researches. Three groups of cows were formed to clarify that in colostrum with different immunoglobulin concentration the content of proteins is different (Table 1). By using Turbidimetric method to determine immunoglobulin concentration among groups, significant differences were not stated, and 1st group amount of immunoglobulin concentration was 4 mg mL⁻¹ more than 3rd group.

Table 1

Immunoglobulin concentration and protein content in colostrum of different cows groups

Groups	Ig concentration, mg mL ⁻¹ , used by Colostrometer	Average Ig concentration, mg mL ⁻¹	Ig concentration, mg mL ⁻¹ , used by Turbidimetric method	Protein content, %
1.	50 – 80	67.9 ± 3.61 ^a	20.0 ± 2.66	12.4 ± 0.84 ^a
2.	81 – 100	87.1 ± 1.68 ^b	17.3 ± 1.37	15.7 ± 0.96 ^b
3.	101 - 123	110.7 ± 3.60 ^c	16.0 ± 1.78	18.2 ± 0.52 ^c

a, b, c – averages of immunoglobulin concentration and protein content with different superscripts differ significantly ($p < 0.05$)

Poorly negative correlation ($r = -0.2$) between immunoglobulin concentration and content of proteins, were observed in colostrum. Colostrum proteins are a significant source of nutrients for new – born calves, and colostrum is a great source of energy (Ontsouka, Bruckmaier, 2003). To determine the content of proteins in colostrum samples, there were observed significant differences among groups of cows ($p < 0.05$). In colostrum samples where the content of proteins was 12.4%, immunoglobulin concentration was 20.0 ± 2.66 mg mL⁻¹ the poor correlation ($r = 0.4$) was observed between results. In samples with the content of proteins 18.2% , immunoglobulin concentration was 16.0 ± 1.78 mg mL⁻¹ and between results there were poor correlation ($r = -0.7$). By using Turbidimetric method to determine immunoglobulin concentration in colostrum, it was clarified that common immunoglobulin concentration in colostrum one week after freezing was 17.9 ± 1.17 mg mL⁻¹, but it was higher 42.7 till 98.9 mg mL⁻¹ in other scientists researches (Morin et al., 1997; Elfstrand et al., 2002).

It is confirmed in researches that cows age influences immunoglobulin concentration in colostrum (Liu et al., 2009). Three groups of cows were formed to clarify if cow's lactation influences a quality of colostrum (Table 2).

Table 2

Immunoglobulin concentration and protein content in colostrum of different lactation cows

Lactation group	Average lactation	Protein %	Ig concentration, mg mL ⁻¹ before storing	Ig concentration mg mL ⁻¹ before storing
			By Colostrometer	By Turbidimetric method
1.	1.7 ± 0.15^a	14.6 ± 0.74	82.7 ± 7.35	19.8 ± 2.05
2.	3.8 ± 0.30^b	15.5 ± 1.01	89.07 ± 4.38	16.7 ± 1.34

a, b – averages of cows lactation with different superscripts differ significantly ($p < 0.05$)

Groups of cows were with different length of usage ($p < 0.05$). Obtained results confirm, that the content of proteins and immunoglobulin concentration between groups do not differ significantly. Negative correlation ($r = -0.1$) was stated by using Colostrometer and Turbimetric method to determine immunoglobulin concentration.

In the first group of cows colostrum the average immunoglobulin concentration was $82.7 \pm 7.35 \text{ mg mL}^{-1}$, but in the second group it was 6.3 mg mL^{-1} more, it complies to other scientists results, it means, that older cows have higher immunoglobulin concentration in colostrum (Kehoe et al., 2011).

To estimate the quality of colostrum by using Turbidimetric method, for the first group it was $19.8 \pm 2.05 \text{ mg mL}^{-1}$, that was 3.1 mg mL^{-1} more than second group. Results are contradicted with other scientists researches. To estimate changes in immunoglobulin concentration in frozen colostrum, it was stored for six months. When colostrum was stored frozen for six months and thawed, immunoglobulin concentration was $10.2 \pm 0.68 \text{ mg mL}^{-1}$ (Figure 1).

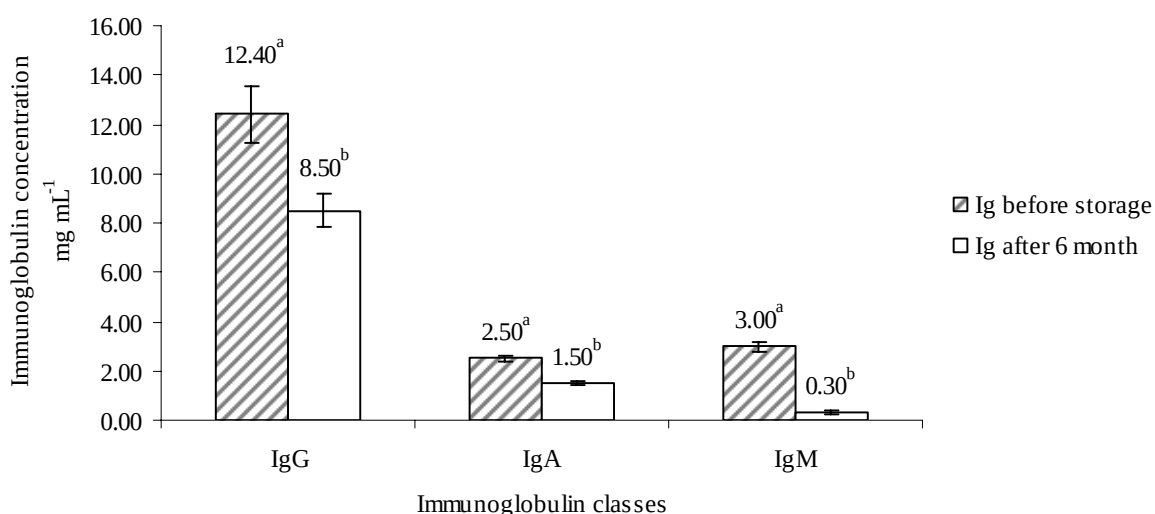


Figure 1. Changes in the concentration of immunoglobulins stored frozen colostrum

a, b – averages of immunoglobulin concentration with different superscripts differ significantly ($p < 0.05$)

After six months the total immunoglobulin concentration decreased significantly to 7.7 mg mL^{-1} or 43%. By analysing separate immunoglobulin concentration, it was observed, that immunoglobulin M decreased fast for 2.7 mg mL^{-1} or 90%, immunoglobulin A decreased for

1.0 mg mL^{-1} or 40%. Immunoglobulin G was the most stable, it decreased for 3.9 mg mL^{-1} or 32%. It is confirmed in researches that immunoglobulin G has a significant role to provide calf passive immunity (Fowden et al., 2006).

Other scientists advice to store colostrum not longer than one year, because immunoglobulin concentration decreases significantly (Quigley, 2001). It is confirmed in researches that by feeding calf with high immunoglobulin concentration in colostrum, it provides calf passive immunity (Jeanne et al., 1989).

CONCLUSIONS

Average immunoglobulin concentration in colostrum that was determined with Colostrometer was $88.2 \pm 3.67 \text{ mg mL}^{-1}$ and it had close connection with the content of proteins in colostrum ($r = 0.8$).

By estimating colostrum with Turbidimetric method, it was stated, that immunoglobulin concentration was $17.9 \pm 1.17 \text{ mg mL}^{-1}$ and its connection with the content of proteins was poorly negative ($r = -0.2$).

Total immunoglobulin concentration determined with Turbidimetric method was low, but the analyse of immunoglobulin G (67%), A (15%) and M (18%) complies to other scientists results.

Immunoglobulin concentration in colostrum decreased 1.7 times after six months storage.

REFERENCES

- Barrington M., Hosteler E., Tyler W., VanMetre C., Weaver M. (2000) Passive transfer colostral immunoglobulins in calves. *Journal of Veterinary Internal Medicine*, Vol. 14, pp. 569 – 577.
- Barrington M., Parish S.M. (2001) Bovine neonatal immunology” in. Immunology. J.A. Roth (ed.) The Vet. Clinics of N.Am. Vol. 17. pp. 463-476.
- Biogenics – Colostrometer (1980) Available at: <http://www.colostrometer.com/support.asp?ID=2> 11 February 2011.
- Coico, R., Sunshine, G., Benjamini, E. (2003) Immunology: a short course. 5. edition. John Wiley & Sons, New Jersey. www3.fmvz.usp.br/..exemploderesumo.
- Dowlen H. H., Martin K. R., Quigley J. D. (1995) Concentration of trypsin inhibitor and immunoglobulin in colostrum of Jersey cows. *Journal of Dairy Science*, Vol. 78, pp. 1573 – 1577.
- Elfstrand L. (2002) Immunoglobulins, growth factors and growth hormone in bovine colostrum and the effects of processing. *International Dairy Journal*, Vol. 12, pp. 879 - 887.
- Elizondo – Salazar J. A., Jayarao B. M., Heinrichs A. J. (2010) Effect of heat treatment of bovine colostrum on bacterial counts, viscosity and immunoglobulin G concentration. *Journal of Dairy Science*. Vol. 93, pp. 961 – 967.
- Fleenor W.A. and Stott G. H. (1980) Hydrometer test for estimation of immunoglobulin concentration in bovine colostrum. *Journal of Dairy Science*, Vol. 63 pp. 973-977.
- Fowden A. L., Ward J. W., Woodling F. (2006) Programming placental nutrient transport capacity. *Journal of Physiology*. Vol. 572. p. 5 – 15.
- Georgiev I. (2008) Differences in chemical composition between cow colostrum and milk. *Bulgarian Journal of Veterinary Medicine*, Vol. 11, pp. 3 – 12.
- Giles F., Baillargeon P., Higgins R., Pare J., Fostin M. (2002) Bacterial concentration of colostrum fed to newborn calves in Quebec dairy herds. *Canadian Veterinary Journal*, Vol. 43, No. 7, p. 523 – 527.
- Jeanne L., Burton B. W., Kennedy E. B., Burnside B. N., Wilkie B.N., Burto J. H. (1989) Variation in serum concentrations of immunoglobulins G, A and M in Canadian Holstein – Friesian calves. *Journal of Dairy Science*, Vol. 72, p. 135 – 149.
- Jones L. R., Taylor A. W., Hines H. C. (1987) Characteristics of frozen colostrum thawed in a microwave oven. *Journal of Dairy Science*, Vol. 70. pp. 1941 - 1945.

Kehoe S. I., Heinrichs A. J., Moody M. L., Jones C. M., Logan M. R. (2011) Comparison of immunoglobulin G concentration in primiparous and multiparous bovine colostrum. *Professional Animal Scientist*, Vol. 27, No. 3, pp. 176 – 180.

Korhonen H., Marnila P., Gill H. S. (2000) Milk immunoglobulins and complements factors. *British Journal of Nutrition*. Vol. 84, No. 1, pp. 75 – 80.

Liu G. L., Wang J.Q., Bu D. P., Cheng J. B., Zhang C.G., Wei H. Y., Zhou L. Y., Zhou Z.F., Hu H., Dong X.L. (2009) Factors affecting the transfer of immunoglobulin G₁ into the milk of holstein cows. *The Veterinary Journal*, Vol. 182, pp. 79 – 85.

Merricks, (2005) Colostrum – the key to calf survival. Available at;

<http://www.merricks.com/pdf/colostrum.pdf>, 3. Marth 2013.

Morin D. E., McCoy G. C., Hurley W. L. (1997) Effect of quality, quantity and timing of colostrum feeding and addition of dried colostrum supplement on immunoglobulin G₁ absorption in Holstein bull calves. *Journal of Dairy Science*, Vol. 80, pp. 747 – 753.

Ontsouka C. E., Bruckmaier R. M. (2003) Fractioned milk composition during removal of colostrum and mature milk. *Journal of Dairy Science*, Vol. 86, pp. 2005 – 2011.

Quigley D. J., Martin K. K., Dowlen H. H. (1995) Concentration of trypsin inhibitor and immunoglobulins in colostrum of Jersey cows. *Journal of Dairy Science*, Vol. 78, No. 7, p. 1573 – 1577.

Quigley, J. (2001) A primer on colostral immunoglobulin. Calf Notes. Available at: [http://](http://www.calfnotes.com)

www.calfnotes.com, 20 January 2013.